INFECTIOUS CAUSES OF INFERTILITY IN BUFFALO BULL (*Bubalus bubalis*)

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ABSTRACT

The artificial insemination (AI) technique plays an important role in improvement of conception rate, prevention of sexually transmitted diseases and transmission of genetic material to the next generation. The breeding soundness evaluation (BSE) plays an important role in selection of male buffalo for the said purposes. This evaluation is an effective, inexpensive and easy method for selection of breeding buffalo bulls. In buffalo breeding management programmes, seasonal variation, nutrition, congenital defect, hormonal changes and hereditary play critical roles in determining the reproductive efficiency in buffalo bulls. The information regarding the reproductive health of buffalo bulls for breeding soundness evaluation is meager. The venereally transmitted infection causes early embryonic death, infection in the female reproductive tract and infertility or sterility in female animals and also infertility and disease condition in the male animals. To avoid such infective conditions, proper and careful investigation of all parts of the reproductive tract and evaluation of semen and treatment are important. So, a male buffalo calf being purchased for breeding purpose must be evaluated for breeding soundness.

Keywords: buffalo bull, *Bubalus bubalis*, infertility, infectious diseases

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 habitats and chromosome numbers. There are about ~170 million buffaloes in the world (Perera *et al.,* 2005). Out of this 97 percent of them are water buffaloes and are mainly found in the Asian region. Riverine buffaloes are characterized by black colour and have long curled horns (e.g. Murrah Breed) and the 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 percent of the total world population) are reared in high numbers in South Asia, especially in India and Pakistan. The name 'swamp' has probably arisen from their preference for wallowing in stagnant water pools and mud holes (Subasinghe *et al.,* 1998). Swamp buffaloes are found mainly in southern China Sri Lanka, and the South-East Asian

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countries of Thailand, the Philippines, Indonesia, Vietnam, Burma (Myanmar), Laos, Cambodia and Malaysia (Chantalakhana and Falvey, 1999). Riverine buffaloes are predominantly used for milk production and they are also used for meat, fuel and fertilizer production, as well as for draught power, whereas swamp buffaloes are traditionally kept as draught animals and are also (to a lesser extent) used for meat production. The river-type buffalo has a diploid number of 50 as compared to 48 for the swamp-type buffaloes. The Murrah has two extra acrocentric chromosomes, which are presumably translocated onto the short arms of the number 1 autosome of the swamp buffalo. Differential staining revealed that Egyptian water buffaloes, with a diploid number of 50, are closely related to the Murrah buffalo (Cribiu and Obeidah, 1978). Hybridization between Murrah and swamp buffaloes is possible and the hybrids are fertile. Improvement of buffalo reproduction helps in production and enhances significantly the economy and living standards of many rural communities throughout the world. The common defects affecting the fertility of the male are congenital defects in the male reproductive tract, hormonal imbalances between different glands, infectious diseases of the reproductive system or any other system, hereditary defects, imbalanced nutrition, psychological defects and abnormal climatic conditions. The purpose of this paper is to describe the different types of infectious disturbance which affect the reproductive performance of the buffalo bull.

GENERAL VIEW OF INFECTIOUS DISEASES IN BUFFALO BULLS

Infectious microorganisms affect fertility in two ways: they directly affect the reproductive system and the quality of semen, and they affect other systems, and this interferes with semen production, affects the libido and mating ability and prolongs reaction time. Lagerlof (1934) established a relationship between semen charactertics and testicular pathology. The condition of the 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 defence against bacteria in the semen, and this in turn leads to the generation of ROS (Ochsendrof, 1998) and also the production of a greater number of dead sperm, which will enhance the production of ROS in semen (Aitken, 1995). Increased ROS generation impairs sperm function and fertilizing capacity (Aitken, 1995; Griveau *et al.,* 1995). Various types of microorganism cause conditions like balanitis, posthitis, seminal vesiculits, prostatitis, urethral inflammation, testicular degeneration, orchitis, epididymitis, and ampulitis which result in male infertility. Under practical conditions, it is not possible to produce semen free from microorganisms as contamination with few nonpathogenic organisms is unavoidable (Binda *et al.,* 1994). Commensal microorganisms also infect the reproductive tract and affect sperm motility and viability when increasing stress to the animal; such organisms include *E. coli* (Schirren and Zander, 1966; Huwe *et al.,* 1998) and *Streptococcus faecalis* (Bisson and Czyglick, 1974; Makler *et al.,* 1981; Huwe *et al.,* 1998).

The organisms present in semen may be bacteria, viruses, protozoa, chlamydia, rickettsia and fungi and generally they are classified as pathogenic, potentially pathogenic, and not pathogenic (Gangadhar *et al.,* 1986). Their

presence in the semen may be due to from the bull's systemic or local specific infections and they 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 storage 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 exposure to the bactericidal action of the vaginal and cervical secretions produced during estrus.

The mean microbiological load was increased by four fold in the second ejaculate (Gangadhar *et al.,* 1986), hence use same artificial vagina for two successive ejaculates is not accepted 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 contaminate approximately 50 percent of frozen semen doses as reported by Wierzbowski *et al.* (1984). Moreover it has been reported that the frozen semen of the buffalo bulls has a higher microbial load (Shukla, 2005 ($4.860 \pm 0.73 \times 10^2$ ml^{-1}); Rathnamma *et al.* (1997) ($5.05 - 171.4 \times 10^3$ ml^{-1}); Jaisal *et al.* (2000) ($1.0 - 50.0 \times 10^2$ ml^{-1}) and there is a highly significant negative ($P<0.01$) correlation of standard plate count with progressive sperm motility (Shukla, 2005), live sperm (Shukla, 2005; Ahmad 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 SPC and type of microorganism vary among different bulls (Shukla, 2005). The common microorganisms isolated from buffalo bull semen are *Pseudomonas sp., Streptococcus* sp., *Staphylococcus* sp., *E.coli*, *Bacillus* sp., *Aeromonas* sp., and yeast (Ghanghadar *et al.,* 1986; Ramasamy *et al.,* 2002). 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 microorganisms has also been reported for buffalo semen in fresh and frozen-thawed semen (Singh *et al.*, 1992; Ramasamy *et al.,* 2002). Microorganisms Balakrishnan *et al.* (2006) and Prabhakar *et al.* (1993) isolated from frozen 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) 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 mcg per ml), chloramphenicol sodium succinate (500 mcg per ml) or ampicillin sodium (500 mcg per ml) in Tris egg yolk glycerol proved to be more beneficial rate than combination of streptomycin sulphate (500 mcg per ml) and penicillin G sodium (500 IU per ml) at every stage of cryopreservation. Gentamicin sulphate was most effective in controlling bacteria in semen at all stages of deep freezing. Gentamicin, being poorly soluble in lipids, has limited ability to cross cell barriers (Prescott and Baggot, 1988). Consequently, its distribution in the extended semen is primarily extra spermatozoal and the whole of

the drug is available for killing bacteria and this is might be the reason for its better bactericidal effect. The better efficacy of chloramphenicol sodium succinate and ampicillin sodium than that of streptomycin sulphate and penicillin sodium G might be due to their higher sensitivity to bacterial flora of semen (Sharma *et al.,* 1994).

INFECTIOUS BOVINE RHINOTRACHEITIS (IBRT)

The disease is caused by Bubaline Herpes Virus 1 (BuHV-1) and Bovine Herpes Virus 1 (BoHV-1), which are members of the Alphaherpesvirinae. BHV-1 (Gibbs and Rweyemamu, 1977) and which infect the respiratory and genital tracts of cattle and buffaloes, causing various diseases, such as 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 are pathogens 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, therefore, 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 test 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 and ocular and reproductive secretions, with the latter route seeming to be the most important for 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 Oirscholt *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 response; therefore, only sero-negative and viruses-free bulls can be used for artificial insemination. There are differences in susceptibility to the disease among breeds. I It has been reported that the Murrah breed (85.8%) is more susceptible ($p = 0.0004$) than the Mediterranean type (67.7%). Moreover, age is also a major factor for susceptibility to this disease: rates of infection increase progressively with advancing age (Ferreira *et al.,* 2010). 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).

FOOT AND MOUTH DISEASES (FMD)

Foot and mouth disease 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 in freezing procedures in liquid nitrogen. Foot and mouth disease causes severe degeneration of germinal epithelium of the testes of bulls. The semen picture was severely adversely affected. The motility was poor and the total sperm abnormalities range 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).

BOVINE VIRAL DIARRHOEA (BVD)

This disease is caused by Bovine Viral Diarrhoea Virus (BVDV) and the virus belongs to genus Pestivirus and family Togaviridae (Andrews *et al.,* 1978). Although cattle are the primary hosts, BVDV can infect most even-toed ungulates. The main mode of transmission is through the oral route, but the disease may also be transmitted by inhalation and artificial insemination using semen of persistently infected bulls. Interspecies spread of this virus has been demonstrated but the epidemiological significance of this is uncertain. Seroepidemiological studies seem to indicate that these viruses circulate to some extent within the buffalo population in Italy (Galiero, 2007). To 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 made it possible 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, thus bringing to light a previously unknown health problem for the buffalo (Galiero, 2007). There are too little 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.

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). Brucellosis is a contagious infectious disease that affects bovines of traditional domestic and nondomestic species such as the buffalo. It is characterized by the production of abortions in the last third of gestation, retention of placenta, metritis, infertility, stillbirth , mastitis, poor production and quality of milk. Affections of the male are arthritis, orchitis, and epididymitis Brucellosis is a zoonosis of world importance (Radostits *et al.,* 2000). In this regard, artificial insemination represents a good tool to control this pathology on condition

that the donors are carefully monitored. Since infected bulls may give serologically negative results, bacteriological tests are necessary. The tests have to be applied to the semen and carried out in successive stages. The seminal plasma has to undergo the serum-agglutination test. Freezing procedures for the preservation of embryos 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 microorganism and the national eradication schemes are more beneficial in eradicating this organism when based on the detection and slaughter of infected buffaloes.

ARCANOBACTERIOSIS

Arcanobacterium pyogenes is commonly present on the nasopharyngeal mucosa of buffalo; in 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 *Arcanobacterium pyogenes* is an important cause of orchitis, epididymitis or seminal vesiculitis, so the organism can be eliminated with semen. Specimens suitable for diagnostic laboratory procedures include exudates, aspirates, tissue samples and semen. *Arcabacterium pyogenes* is a Gram positive pleomorphic rod and produces a characteristic haemolytic pin-point colony in 48 hours of incubation. The diagnosis is based on bacteriological examination of the organs. *Arcanobacterium pyogenes* develops in 24-48 h, forming haemolytic colonies on agar supplemented with 5% sheep erythrocytes. Biochemical tests yield definitive microbial identification (Galiero, 2007).

CAMPYLOBACTERIOSIS

The infection of buffaloes with C*ampylobacter foetus* may be widespread. *Campylobacter fetus* subsp. *venerealis* causes a type of venereal disease in the cow that is transmitted through natural service or artificial insemination. The disease is characterized by infertility, prolonged estrous, premature embryo death and in some instances, untimely abortion with placental retention (Das and Paranjape, 1987). The use of communal bulls and the use of males that have not been tested for C*ampylobacter foetus* at artificial insemination centers are important factors in spreading infection. It has been reported that there are seven strains of *Campylobacter sputorum* subsp. *Bubulus* (Modulo *et al.,* 1997) and *Campylobacter fetus*, *Campylobacter fetus* subsp. *venerealis* and *Campylobacter fetus* subsp. *fetus* (Joshi *et al.*, 2006) have isolated from the prepuce of buffalo bulls. The disease has been recorded in India, Malaysia and the former U.S.S.R. (Eaglesome and Garcia, 1992). The animals used for artificial insemination have to undergo quarantine and have negative results in three consecutive cultural tests carried out on preputial scraping. Afterwards their sanitary conditions have to be checked every six months.

LEPTOSPIROSIS

The microorganisms belonging to this genus are mobile, helical bacteria, the terminal part of the bacterial body being hooked-shaped. Although cytochemically Gram-negative, they do not stain

well with the conventional bacterial stains, and are normally observed under a dark-field microscope. In the past, leptospires were subdivided 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 tract. The pathogenic role of *Leptospira 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 microorganisms cause hypofertility and abortion and survive in the frozen semen, particular attention has to be paid to 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 have also been 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.

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 hypofertility. *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 microorganism 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.

MYCOBACTERIOSIS

Mycobacterium bovis can be responsible of orchitis in the buffalo male. Therefore, the donor buffalos' 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 the gamma interferon test, ELISA and PCR.

TRICHOMONIASIS

Trichomoniasis is caused by *Trichomonas foetus,* a pyriform protozoan that causes premature abortions, pyometra and infertility. This flagellate is transmitted to the female buffalo during her mating 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 persists in diluted semen and is resistant to freezing; therefore, donors have to be tested frequently either through a microscopic examination or through a cultural test in order to exclude any infection.

MYCOPLASMOSIS

Various workers have reported the recovery of mycoplasma from bovine semen. It is established fact that mycoplasma is common in the bull and buffalo bull semen and survives during semen processing, freezing and storage. The survival of mycoplasma in frozen semen at -196^{o}C for 18 months has been reported. Mycoplasma cause granular vulvo-vaginitis and damage the inner lining of the oviduct in females while impairing fertility in males. Mycoplasma especially the *bovigenitalicum* and *agalactiae* species are frequently associated with seminal vesiculitis in the male and metritis and salpingitis in the female.

FUNGI (MOULD AND YEAST) 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 conditions. 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 primarily by culture isolation and *Aspergillus* sp., *Fuzerium* sp., *Penicillum* sp., *Mucor* sp., 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 animals liable for mycotic infection.

Escherichia coli INFECTIONS

E. coli is a gram-negative, non-endospore producing, 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. To 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 and producing 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).

OTHER MISCELLANEOUS MICRO ORGANISMS

The other micro organisms that are involved in infectious infertility in buffaloes are *Usteria monocytogenes, Pseudomonas aeruginosa, Bacillus licheniformis, Streptococcus* spp., *Staphilococcus* spp., *Proteus* spp., *Aeromonas* spp., yeast and moulds. Fungal infections may develop during the processing of semen for preservation. Fungal infections of the prostate and other accessory sex glands may result in the 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 the prepuce and upper parts of the genital organs. Since the greatest amount of the bacteria present in semen comes from the prepuce, from the skin and from the equipment used, semen collecting procedures have to be carried out using sterilized tools and materials and the bull's preputial cavities must be cleaned constantly. In spite of these preventive measures, sometimes the ejaculate presents a non-specific microbial flora (5×10^6 CFU/ml). These concentrations may be reduced by administering antibiotics and diluting the semen, whihc lower the number of viruses and bacteria about 100 times, and freezing until the following count is obtained: 8×10^2 CFU/ml (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 1000 to 5000 count) should carry lowest minimum bacterial load with no count for AI equipment 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°C in liquid nitrogen (Ranold and Prabhakar, 2001).

FUTURE PROSPECTS

The future prospects to improve the reproductive traits and eliminate the reproductive disorders in buffalo bulls are mentioned below.

There is a need to study to a greater extent the sexual development, attainment of puberty, sexual maturity, sex libido, semen production, reproductive performance of buffalo bulls. Study of molecular marker (DNA/gene) assisted selection and cytogenetic studies linked to genes of interest with major effects on reproduction need to be strengthened for selection of breedable males in bubaline species at an early age. A cytogenic marker would help to cull/eliminate a particular bull from the herd to prevent the spread of a genetic/ congenital defective gene from that male to future offspring and to prevent reproductive disorders.

Breeding soundness evaluation technique would help to improve the breeding efficiency of buffalo bulls.

Realistic remedial measures for reducing infertility and enhancing fertility need to be emphasized for the effective control of various reproductive disorders.

Biosafety measures for production disease-free germplasm and registration of all A.I. bulls by a national society to initiate a certified disease-free semen service for the whole nation need to be addressed.

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