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## EVIDENCE OF BACTERIAL CONTAMINATION IN THE FROZEN BOVINE SEMEN

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### ABSTRACT

Artificial insemination (AI) has been a successful method that is used for the breeding of domestic animal species around the globe. However, the semen may get contaminated with microbial agents during processing and storage of the semen and may cause infertility and/or local infections in the genital tract. The bacterial contamination of frozen semen from local centers was investigated. The findings revealed that out of 100 frozen semen samples, 7 were found positive for the various bacterial isolates, while 93 were negative without any microbial growth. The positive samples examined, 5 (71.42) and 2 (28.57%) contained pure and mixed bacterial species. The identified bacterial species, *Acinetobacter* 1 (11.11%), *Actinobacillus lignerisi* 2 (22.22%), *Citrobacter* 1 (11.11%), *Micrococcus luteus* 1 (11.11%), *Pseudomonas aeruginosa* 2 (22.22%), *Staphylococcus epidermidis* 1 (11.11%) and *Staphylococcus intermedius* 1 (11.11%) were present in frozen bovine semen samples. The findings of this study suggested the occurrence of bacterial species in the frozen bovine semen. Therefore, the frozen bovine semen should be screened out for microbial contamination before use for artificial insemination.

**Keywords:** Artificial insemination, bacteria, bovine, contaminants, frozen semen.

### INTRODUCTION

Artificial insemination (AI) has been a successful technique that is used for the breeding of cattle and other domestic animal species around the world. The method is a valuable tool that benefits breeders to gain high quality genetic potential from proven bulls (Verkerk, 2003; Funk, 2006). There is increasing trend of artificial insemination, approximately 150 million cows have been artificially inseminated (Bonadonna and Succi, 1980). It has been estimated that

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243 million doses of bovine semen are produced annually (Thibier and Wagner, 2000; Givens and Marley, 2008).

Routinely, semen is packaged in straws approximately 0.25 ml or 0.5 ml, pellets and flatten plastic bags for freezing and storage. The frozen straws and flattened plastic bags are transported in liquid nitrogen for the artificial insemination (Bwanga *et al.*, 1991; Weitze *et al.*, 1991). However, there is a risk of contamination of semen from pathogens during the packaging and storage that can adversely affect the fertility and reproductive efficiency (Russell *et al.*, 1997). In general, fresh semen or every ejaculate contains some of nonpathogenic microbial contaminants that are not detrimental for the artificial insemination. However, the excessive load of these microbial agents may result in infertile matings (Thacker *et al.*, 1984). The semen may get contaminated with pathogenic and non-pathogenic microbial agents during processing and storage of the semen. These microbial agents gain access to the semen and can transfer serious diseases in recipient farm animals. This may lead to bacteraemia, viraemia and local infections in different parts of genital tract (Thibier and Guerin, 2000).

Several studies evaluated the contamination of bacterial pathogens such as *Acinetobacter cacloaceticus*, *coxiella burnetti*, *Escherichia coli*, *Flavobacterium* species *Pantoeau agglomerans*, *Corynebacterium spp*, *Staphylococcus aureus*, *Micrococcus*, *Leptospira spp*, *Histophilus somni*, *Enterobacter cloacae*, *Brucella suis*, *Ureaplasma diversum*, *Stenotrophomonas maltophilia*, *Enterobacter-coccus*, *Staphylococcus sciuri*, *Chlamydophila abortus* and *Pseudomonas aeruginosa*, in the frozen semen of farm animals (Ramaswamy *et al.*, 1990; Ramaswamy *et al.*, 1994; Kruszewska and Tylewska-Wierzbanowska, 1997; Thibier and Guerin, 2000; Ramaswamy *et al.*, 2002; Bielanski *et al.*, 2003; D'Angelo *et al.*, 2006; Schlafer and Miller, 2007; Vinodh *et al.*, 2008; Corona and Cherchi, 2009; Hobson *et al.*, 2013). Also the presence of a number of viruses such as bovine viral diarrhea virus, foot and mouth disease virus, Bovine enterovirus, bluetongue virus, infectious bovine rhinotracheitis virus, bovine leukemia virus, lumpy skin disease virus, parapoxvirus and ephemeral fever virus have been found in bull semen (Kahrs *et al.*, 1980). In addition to contamination with bacteria and viruses, fungi and other microbial agents have been reported in the frozen semen of cattle. Considering the situation, it is important to analyze frozen semen for the population of microorganism to obtain successful artificial insemination in the farm animals. Therefore, the present study was designed to evaluate the bacterial contamination in the frozen semen of cattle in order to prevent any introduction of diseases and to achieve better fertility rate.

## **MATERIALS AND METHODS**

One hundred frozen semen samples of cattle were collected under sterile hygienic condition from the local semen production centers. The semen samples were contained in straws and sterilized bijoux bottles in artificial insemination kits, which contained liquid nitrogen and brought to the laboratory. Different

dehydrated media were used for the culture or presence of any bacteria in the frozen semen samples. Dehydrated nutrient agar (Difco, 2000), MacConkey agar (Difco, 2000) and blood agar (Difco, 2000) were rehydrated according to recommendation of manufacturer. The media were stirred to dissolve and then autoclaved at 121°C under 15 lb pressure for 15 min. Cooled and blood agar at 45-50°C was added with 5% defibrinated aseptically sheep blood. The samples were inoculated by streaking method on nutrient, blood and MacConkey's agar media and incubated aerobically at 37°C for 24 h for the presence of microbial agents. The bacterial colonies that were grown on the media were sub-cultured to achieve pure culture of bacteria. The single colony was taken for the preparation of smear and routine staining procedure. The cultured bacteria were observed for morphological characteristic.

Further the colonies were taken for the pure culture and for the biochemical properties and sugar fermentation tests. Different biochemical tests such as catalase, coagulase test, gelatin liquefaction, aesculin test, bile tolerance test, Hugh and Leifson's test, indole production test, methyl red, methyl blue Proskauer test oxidase, triple sugar iron agar, Simmon's citrate, urease production test, nitrate reduction and sugar fermentation tests were performed as prescribed by Khalil and Gabbar (1992); Christensen *et al.* (2002); Abro *et al.* (2009). These biochemical and sugar fermentation tests were performed for the identification and confirmation of the isolates contained in the frozen semen samples.

## RESULTS AND DISCUSSION

In this study, one hundred frozen semen samples of cattle were collected from local semen production units and these samples were examined for the contamination or presence of bacterial species. The findings revealed that out of 100 frozen semen samples, 7 were found positive for the various bacterial isolates, while 93 were negative without any bacterial growth (Tables 1-2). It has been reported that contaminated fresh and frozen semen used for artificial insemination spread the diseases in the animals and also adversely influence fertility and reproductive efficiency (Thacker *et al.*, 1984; Russell *et al.*, 1997).

Therefore, it is important to evaluate the frozen semen before use for artificial insemination in order to prevent introduction of the disease and/or risk of microorganisms in the farm animals. The bacterial species identified from the frozen semen samples during the study are given in Table 3. The 100 samples examined, 5 (71.42) and 2 (28.42%) were determined having pure and mixed bacterial species (Table 2). The present study demonstrated that bacterial species, *Acinetobacter*, *Actinobacillus ligneirisi*, *Citrobacter*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Staphylococcus intermedius* were present in frozen semen sample either individually or in association with other bacterial species (Tables 2-3). To the results of this study, there are reports on the occurrence of the certain bacterial species in the frozen semen used for the artificial insemination. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were found in the frozen semen of cattle (Akhter *et al.*,

2008). Similarly, *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus* and *Corynebacterium spp* have been identified in frozen semen of cattle (Ramaswamy *et al.*, 1990; Ramaswamy *et al.*, 1994; Ramaswamy *et al.*, 2002). Corona and Cherchi (2009) had screened equine frozen semen for the presence of microorganisms and found *Acinetobacter spp.*, *Staphylococcus spp.* and *Pseudomonas spp.* in the samples. The findings regarding the presence of *Pseudomonas aeruginosa*, *Micrococcus*, *Acinetobacter* and *Staphylococcus species* in the frozen and fresh semen samples are in accordance with the previous reports (Ramaswamy *et al.*, 1990; Ramaswamy *et al.*, 1994; Ramaswamy *et al.*, 2002; Corona and Cherchi, 2009). The results are further providing the evidence that the microbial contaminants may be found in fresh and frozen semens used for the artificial insemination. However, the sources of these microorganisms need to be verified through future studies.

Table 1. The prevalence of bacterial species isolated from frozen semen of cattle.

Animal species	No. of frozen semen samples examined	No. of positive frozen semen samples	Percentage	No. of Negative frozen semen samples	Percentage
Cattle	100	7	7	93	93

Table 2. The prevalence of pure and mixed bacterial species isolated from frozen semen of cattle.

Animal species	No. of samples examined	% of positive samples	No. of pure positive samples	% of pure positive samples	No. of mixed positive samples	% of mixed positive samples
Cattle	100	7	5	71.42	2	28.42

Table 3. The prevalence of pure and mixed bacterial species isolated from frozen semen of cattle.

Bacterial species	No. of samples occurring in	Percentage
<i>Acinetobacter</i>	1	11.11
<i>Actinobacillus ligneirisi</i>	2	22.22
<i>Citrobacter</i>	1	11.11
<i>Micrococcus luteus</i>	1	11.11
<i>Pseudomonas aeruginosa</i>	2	22.22
<i>Staphylococcus epidermidis</i>	1	11.11
<i>Staphylococcus intermedius</i>	1	11.11
Total		100

## CONCLUSION

In conclusion, the occurrence of bacterial contaminants were observed in the bovine frozen semen used for the artificial insemination. Therefore, it is worth, to examine the frozen bovine semen before used for artificial insemination in order to prevent introduction of the disease and/or risk of microorganisms in the farm animals.

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