*The Indian Journal of Animal Reproduction; 26(2): 168-169; December 2005*

Short Communication

# Studies on the microbial load of cryopreserved Murrah semen

M. K. SHUKLA[†], A.K. MISRA[1], H.P. GUPTA[2] AND S.P. SINGH[3]

Department of Animal Reproduction, Gynaecology and Obstetrics,
College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology,
Pantnagar -2631456, U.S. Nagar (Uttaranchal)

Received : September 10, 2003
Accepted : August 17, 2005

## ABSTRACT

The objective of the present study was to determine microbial load in frozen semen of three Murrah bulls using ten ejaculates from each bulls. The average bacterial load determined using pour plate method in the frozen semen of the three bulls was 79.00±0.76, 7.00±0.91 and 60.00±0.87, respectively with an overall mean of 48.60±0.73 colony forming units (CFU) $ml^{-1}$. The SPC differed significantly ($P< 0.01$) between the three bulls. All the semen samples were found to be negative for fungus, mucor and yeast.

**Key words**: Buffalo, semen, microbial load, standard plate count

The bacterial contaminants of semen has been a major concern for most of the semen production laboratories as it adversely affects the semen quality (Diemer *et al.*, 1996) and hence the subsequent fertility (Ochsendrof and Fuch, 1993; Griveau *et al.*, 1995). Certain bacterial contaminants acquire a certain level of resistance to antibiotics and are able to survive at -196°C in liquid Nitrogen (Ronald and Prabhakar, 2001). These bacteria are reported to account for the contamination of approximately 50 % of frozen semen samples (Wierzbowski *et al.*, 1984).

The present study was, therefore, conducted to study the microbial load in the frozen semen. A total of 30 randomly selected ejaculates of three Murrah bulls (10 from each bull) were used in the study. The semen collection was done in a sterilized artificial vagina adopting all aseptic precaution and was preceded by prepucial wash with 0.01 % Acriflavin solution 12 h and 20 min. before semen collection. The semen thus collected was diluted in egg yolk tris glycerol extender and frozen in French in mini straws using horizontal liquid nitrogen vapour freezing technique (Verma *et al.*, 1975) after allowing an equilibration period of 4 h at 4°C. Three frozen semen straws were thawed at 37°C for 1 minute to assess the bacterial load. The content of the straws was evacuated in a sterilized sugar tube after wiping the straws with methanol. The thawed semen samples were subjected to standard plate count using pour plate method (Cruickshank *et al.*, 1975). Two plates for each sample and one control, with each batch, were kept in incubation at 37°C for 48 h. After 48 h of incubation the bacterial colonies were counted with a colony counter.

The thawed semen was also examined for fungus, mucor and yeast by inoculating the samples in Sabaroud's Dextrose agar at 37°C for 24 h followed by subsequent examination of the organism. The results were statistically analyzed using analysis of-variance (Snedecor and Cochran, 1967).

The average microbial load of the frozen semen, assessed by standard plate count, of the bulls, MB I, MB II and MB III was 79.00±0.76, 7.00±0.91 and 60.00±0.87 CFU $ml^{-1}$ which is well within the limits set by Bureau of Indian Standards (BIS) and OIE. The findings of the present experiment may be fairly compared with the previous reports ($1.1x10^2$ $ml^{-1}$ in crossbred bull: Kumar *et al.*, 1994) whereas some other workers have reported significantly higher bacterial load in semen samples ($8.1\text{-}390.4x10^2$ $ml^{-1}$ in frozen semen of Holstein stored for 15 days: Rathnamma *et al.*, 1997; $41\text{-}180x10^2$ $ml^{-1}$ in buffalo semen: Jaisal *et al.*, 2000). In a study on buffalo semen (Ramaswamy *et al.*, 1997), 58% frozen semen samples were contaminated with 23 microorganisms including 2 yeasts. Remarkably low bacterial load of the frozen semen samples in the present study may be due to prepucial wash and maintenance of strict aseptic conditions at the time of semen

[1]Professor
[2]Associate Professor
[3]Professor, Dept. of Vety. Public Health

[†]Corresponding author

collection and processing. A highly significant ($P<0.01$) difference between the standard plate count of the three bulls was observed in the present study, which is in confirmation with the findings of Ahmed and Mohan (2001). The semen samples from all the three bulls were found to be negative for fungus, mucour and yeast.

## REFERENCES


Ahmed, K. and Mohan, G. (2001). Effect of antibiotics on the bacterial load and quality of semen of Murrah buffalo bulls during preservation. Ind. J. Anim. Reprod., **22**: 79-80.

Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. (1975). *Medical Microbiology*, 12th edn. Churchill Livingstone, Edinburg, pp 170-180.

Diemer, T., Weidner, W., Michelmann, H.W., Sciefer, H.G., Rovan, E. and Mayer, F. (1996). Influence of *Escherichia coli* on motility parameters of human spermatozoa *in vitro*. Int. J. Androl., **19**: 271-277.

Griveau, J.F., Domount, E., Renard, P., Challegani, J.P. and Lelannou, D. (1995). Reactive oxygen species lipid peroxidation and enzymatic defense system in human spermatozoa. J. Reprod. Fertil., **103**: 17-26.

Jaisal, S., Katoch, R.C., Chachara, D. and Mahajan, A. (2000). Evaluation of bacterial load in fresh ejaculates of bovine and buffalo bull semen in Himachal Pradesh. Ind. J. Anim. Sci., **70**: 465-467.

Kumar, A.G., Raju, P.V., Prabhakaran, N.K., Kumar, G.A. and Nair, K.P. (1994). Type of bacteria and its load in fresh semen of cross-bred bulls. Ind. J. Anim. Reprod., **15**: 48-49.

Ochsendrof, F.R. and Fuchs, J. (1993). Oxidative imbalances in male fertility. In: *Oxidative Stress in Dermatology*. Fuchs, Packer, L. (eds.) Marcel Dekker, New York, Basel, Hongkong, pp 489-531.

Ramaswamy, V., Latha, N., Gnanasubramaniam, T. and Venugopalan, A.T. (1997). Occurrence of bacteria in frozen buffalo semen and their antibiogram. J. Vet. Malaysia, **9**: 19-21.

Rathnamma, D., Rao, M.S., Ramanatha, K.R. and Raghavan, R. (1997). Assessment of bacterial load in semen of Holstein Friesian bulls. Curr. Res. Univ. Agri. Sci., Bangalore, **26**: 205-207.

Ronald, B.S.M. and Prabhakar, T.G. (2001). Bacterial analysis of semen and their antibiogram. Ind. J. Anim. Sci., **71**: 829-831.

Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods*. The Iowa State Univ. Press, U.S.A.

Verma, M.C., Saxena, V.B., Tripathi, S.S. and Singh, R. (1975) A note on deep freezing of Murrah and Jersey bull semen. Ind. J. Anim. Sci., **45**: 970- 971.

Wierzbowski, S., Nowakowski, W., Wayda, E and Kuzniak, S. (1984). Antibiotic level and bacterial contamination of frozen bull's semen [streptomycin, penicillin]. Medycyny-Weterynaryjna (Poland), **40**: 284-287.


# OBITUARY

Dr. Abdus Sattar, Ex-Director of Animal Husbandry and Veterinary Sciences, Assam passed away on 2nd August, 2005 after a brief illness. Born on 1st November, 1933 at Titabor of Jorhat district of Assam. Dr. Sattar graduated from Assam Veterinary College, Guwahat in the year 1954 and obtained post graduate diploma in Animal Reproduction from IVRI, Izatnagar. Later on Dr. Sattar was conferred FRCS from Royal Veterinary College, Stockholm, Sweden. He completed P.G. courses in frozen semen technology from Sydney, Australia. He was life member of ISSAR and was elected as the President, Assam Chapter of the Society for 2005 and 2006.