



STUDIES ON ANTIBIOGRAM PROFILES OF FROZEN BULL SEMEN

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Received: 14/05/2018

Edited: 26/05/2018

Accepted: 04/06/2018

Abstract: A total of 34 HF bull semen samples were screened and subjected to total aerobic bacterial load estimation. The bacterial load was estimated for the neat, extended and frozen bull semen samples to assess the bacterial contamination of semen during handling, processing and storage of the semen samples. In the present study, the bacterial load of the frozen semen samples was found to be within the BIS standards.

Key words: Antibioqram Profiles, Bull Semen.

Introduction

The microbes because of their ubiquitous presence have ample access to contaminate the semen during collection, processing and preservation stages. The semen though free from specific pathogens gets contaminated subsequently sometimes even under strict hygienic conditions. Presence of opportunistic pathogenic organisms may compete along with the spermatozoa for nutrients (Salisbury *et al.*, 1978) and may cause reproductive disorders (Eaglesome *et al.*, 1992) when used. The addition of Penicillin and Streptomycin in semen may control the contaminating bacteria, but there are increasing reports of resistance to these drugs by bacteria present in the semen (Rahman *et al.*, 1983; Kumar *et al.*, 1994).

The production of frozen semen of a larger dimension needs a quality control service to maintain supply of good quality frozen semen and to attain a good quality of frozen semen, microbial analysis should be carried out at each and every step of processing and the present study was undertaken to estimate the bacterial load of semen at various stages of semen processing and suggest suitable measures to control the bacterial load. Also, to know the

feasibility of using different antimicrobial agents in reducing the contaminants in the semen.

Materials and Methods

A total of 34 frozen semen samples of HF bulls, procured from Nandini sperm station, Kakolu, Bangalore were used for the present study. The bulls from which the semen samples were collected were in good general and reproductive health and free from any known specific diseases. The in vitro drug sensitivity test was carried out with 6 antimicrobial agents by paper disc diffusion technique (Bauer *et al.*, 1966) using biodiscs (Hi-Media Laboratories, Mumbai, India).

Results and Discussion

In the present study, none of the neat semen samples were negative for bacterial growth. While in extended semen samples, five out of the 36 (13.88%) samples were negative for bacterial growth/ sterile. Out of the 34 frozen thawed semen samples, 29 samples (85.29%) showed growth of bacterial colonies, whereas 7 samples (20.58%) were sterile.

Sensitivity observed to Lincomycin, Spectinomycin, Tylosin, Gentamicin, Amoxyclav, and Enrofloxacin decreased from 86.1% to 27.7% (Table 1).

Table 1: Sensitivity and resistance patterns of bacterial cultures of semen samples to various antibiotics

Antibiotic	Sensitive		Resistant	
	No.	Percent	No.	Percent
Gentamicin	26	76.47%	8	23.52%
Tylosine	27	79.41%	7	20.58%
Lincomycin	29	85.29%	5	14.70%
Spectinomycin	28	82.35%	6	17.64%
Enrofloxacin	9	26.47%	25	73.52%
Amoxyclav	13	38.23%	21	61.76%

Antibiotics are routinely added to the extenders before semen extension to check the bacterial contamination, if any. However, in spite of addition of antibiotics, there have been reports of elevated cfu levels of bacteria in the frozen semen than that after dilution (Hasan *et al.*, 2001). This has been attributed to the antimicrobial resistance in turn due to its indiscriminate usage. In view of such ever-growing multi-drug resistance, introduction of new generation antibiotics for reducing the bacterial load in semen, drug sensitivity of the bacterial isolates of semen is imperative (Sandeep Jaisal *et al.*, 2000b). Current international standards with regard to the antibiotic components of semen extenders have made it necessary to look for alternatives for the Strepto-penicillin containing extender (CSS, 1993; Hasan *et al.*, 2001).

The results of the present study showed that the percentage of resistance to Gentamicin (23.6%) was lower and the observation is in agreement with reports of Sandeep Jaisal *et al.* 2000b. A higher resistance to penicillin and Streptomycin was also observed by Singh *et al.* (1990), Ramaswamy *et al.*

(1990), Gupta and Maurya (1993), Prabhakar *et al.* (1993), Ramaswamy *et al.* (1994), Bindra *et al.* (1994), Sandeep Jaisal (2000b), Ahmed and Greesh Mohan (2001), Ronald and Prabhakar (2001) and Ahmed and Greesh Mohan, (2002).

It may be concluded that bacterial contamination in frozen semen can be minimised by strict hygienic practices even without addition of antibiotics during processing. However, if the use of antibiotics becomes inevitable, conventional antibiotics like- Penicillin and Streptomycin may still be sufficient. As for this study is concerned, only Penicillin and Streptomycin have been used which maintained the bacterial load at 260 cfu/ml of frozen semen which was very well below the permissible level (500cfu/ml) set by the Bureau of Indian Standards. However, in the event of development of antimicrobial resistance, the best results can be obtained by studying the antibiogram of bacterial isolates from semen and changing the antibiotics and their dose for incorporation during processing of frozen semen depending on the results of such study.

References

- AHMED, K. and GREESH MOHAN., 2001. Effect of antibiotics on bacterial load and quality of semen of Murrah bulls during preservation. *Indian J. Anim. Reprod.*, **22**: 78- 80
- AHMED, K. and GREESH MOHAN., 2002. Effect of antibiotics on the bacterial load and quality of semen of Murrah buffalo bulls at different stages of freezing. *Indian J. Anim. Sci.* **72**(2): 138-139
- BAUER, A. W., KIRBY, W.M. M., SHERRIS, S. C. and TURK, M., 1966. Antibiotic susceptibility testing by a standardized single disc diffusion method. *American J. Clin. Pathol.*, **45**:493
- BINDRA, D. S., PANGAWKAR, G. R., DHINGRA, P. N. and MATHAROO, J. S., 1994. Bacterial flora and their antibiotic sensitivity pattern in buffalo bull semen. *Indian Vet. J.*, **71**: 460- 463
- CERTIFIED SEMEN SERVICES (CSS®), 1993. CSS minimum requirement for health of bulls producing semen for AI, Missouri

- EAGLESOME, M. D., GARGIA, M. M. and STEWART, R. B., 1992. Microbial agents associated with bovine genital tract infections and semen. Part II. *Haemophilus somnus*, *Mycoplasma sp.* and *Ureaplasma sp. Chlamydia*; pathogens and semen contaminants; treatment of bull semen with antimicrobial agents. *Vet. Bull.*, **62**:887-910.
- GUPTA, R. S. and MAURYA, S. N., 1993. Microbial flora of bovine semen and their antibiotic sensitivity pattern. *Indian J. Anim. Reprod.*, **14**: 107- 108
- HASAN, S., ANDRABI, S. M. H., MUNEEER, R., ANZAR, M. and AHMAD, N., 2001. Effects of a new antibiotic combination on post-thaw motion characteristics and membrane integrity of buffalo and Sahiwal bull spermatozoa and on the bacteriological quality of their semen. *Pakistan Vet. J.*, **21**: 6-12
- KUMAR, G., RAJU, P.V. and NAIR, P. 1994. Type of bacteria and its load in fresh semen of cross-bred bulls. *Indian J. Anim. Reprod.*, **15**: 48-49
- PRABHAKAR, T. G., PREMKUMAR, D. B., MANOHAR, P, W., VENKATASUBBA RAO, C. and VENKATESAN, R. A., 1993. Bacterial analysis in the frozen semen of buffalo bulls. *Indian J. Anim. Sci.*, **63**: 644- 645
- RAHMAN, A., DUTTA, J. C., BORO, B. R. and RAJKONWAR, C. K., 1983. Studies on bacteriological flora of bull semen and their antibiotic spectra. *Indian Journal of Comparative Microbiology, Immunological and Infectious Diseases.*, **4**(2):110-112
- RAMASWAMY, V., JOHN, K. J., JOSEPH, A. J. M., PARIMAL, R. and VENUGOPALAN, A. T., 1994. Prevalence of microbes in frozen cattle semen and their antibiotic spectra. *Indian J. Anim. Reprod.*, **15**(1): 50-52
- RAMASWAMY, V., SARAVANABAVA, K., ANDREW, M. J., VENUGOPALAN, A. T. and VENKATESAN, R. A., 1990. Microbial flora of buffalo frozen semen and their antibiogram. *Indian J. Anim. Sci.*, **61**: 843- 845
- RONALD, B. S. M. and PRABHAKAR, T. G., 2001b. Bacterial analysis of semen and their antibiogram. *Indian. J. Anim. Sci.*, **71**(9): 829-831
- SALISBURY, G. W., VAN DENMARK, W. C. and LODGE, J. R., 1978. *Physiology of Reproduction and Artificial Insemination of Cattle*. Edn 2nd. W. H. Freeman and co. San Francisco. pp 286- 328
- SANDEEP, J., KATOCH, R. C., DEEPTI, C. and ARVIND, M., 2000b. The drug resistant bacteria associated with frozen semen technology of cow and buffalo bulls. *Indian J. Anim. Sci.*, **70**: 488-489
- SINGH, N., SINHA, A. K., BALRAJ SINGH, SINGH, L. C., TIWARY, B. K. and SINGH, M. P., 1990. Efficacy of specific antibiotics on microbial load in chilled and frozen semen of crossbred bulls. *Indian Vet. J.*, **67**(6):531-536