

### Short Communication

## ISOLATION OF AEROBIC, FACULTATIVE BACTERIA FROM THE SEMEN OF HOLSTEIN FRIESTAN BULLS

Contamination of fresh and frozen semen with microorganisms poses a great threat to successful cattle breeding programme. Even under hygienic conditions semen may get contaminated at the time of collection or subsequent handling. Studies on microbial flora of bovine semen have been done in India by a few workers (Gupta and Maurya, 1993; Bindra *et al.*, 1994). The present study was aimed at isolating bacteria if any from semen of Holstein Friesian bulls.

Twenty seven Holstein Friesian bulls of different ages from three different semen collection centres located in and around Bangalore were used for the study. Semen samples were collected aseptically by means of artificial vagina. A total of 324 semen samples (each 81) including neat, extended, frozen and frozen semen stored for 15 days in liquid nitrogen were screened for the presence of bacteria. The media were prepared according to the methods of Cruickshank *et al.* (1975) and Collie *et al.* (1989). The isolates were identified as per the Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984; Sneath *et al.*, 1986).

A total of 238 gram positive and 102 gram negative bacteria were isolated from various samples collected from different centres (Table 1). There was an obvious difference in the type of bacterial isolates. *Corynebacterium renale* and

*Corynebacterium xerosis* which were not recovered from extended and stored frozen semen though these were recovered from neat and frozen semen from all the three centres. The isolates *Bacillus megatherium*, *Corynebacterium renale*, *Corynebacterium xerosis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Enterobacter aerogenes* and *Citrobacter* spp. were not reported from one centre but these were reported from other two centres. Some of the isolates were not recovered from extended, frozen semen and stored frozen semen. Although the isolation of bacteria from preputial washings was not done, there are reports (Bindra *et al.*, 1994) that many of these Gram positive and Gram negative bacteria were isolated from the prepuce of bulls thereby establishing the prepuce as one of the source for contaminants found in neat semen. The possible explanation for the absence of some of the bacteria in extended and frozen semen could be related to the dilution and freezing and thawing effects, respectively. The presence of the bacteria in the extended semen could be due to the contaminated extender which was used for extending or diluting neat semen, reported by Bindra *et al.* (1994).

The isolation of *Pseudomonas aeruginosa*, *Corynebacterium* species, *Escherichia coli* was significant due to their reported deleterious effects on spermatozoa. *Pseudomonas aeruginosa* have been found to

Table 1 Bacterial isolates (%) obtained from neat, extended, frozen and stored frozen semen from different centres

Type of isolate		Successful isolations of different bacterial species from				
		Neat semen (81)	Extended semen (81)	Frozen semen (81)	Stored frozen semen (81)	Total (324)
<i>Gram positive bacterial</i>	'n'	127	35	52	24	238
<i>Bacillus subtilis</i>		25.98	37.14	23.07	25.00	26.89
<i>Bacillus megatherium</i>		14.17	14.28	9.61	16.66	13.44
<i>Corynebacterium renale</i>		4.71	NR	3.84	NR	3.36
<i>Corynebacterium xerosis</i>		7.87	NR	7.69	NR	5.88
<i>Corynebacterium</i> spp.		6.29	2.85	3.84	8.33	5.46
<i>Micrococcus</i> spp.		16.53	20.00	21.15	29.16	19.32
<i>Staphylococcus aureus</i>		13.38	20.00	19.23	20.83	16.38
<i>Staphylococcus spidermidis</i>		11.02	5.71	11.53	NR	9.24
<i>Gram negative bacteria</i>	'n'	57	8	28	9	102
<i>Citrobacter</i> spp.		5.26	NR	3.57	NR	3.92
<i>E. coli</i>		21.05	NR	21.42	44.44	21.56
<i>Enterobacter</i> spp.		5.26	NR	NR	NR	2.94
<i>Enterobacter aerogenes</i>		15.78	25.00	21.42	NR	16.66
<i>Proteus mirabilis</i>		17.54	25.00	10.71	33.33	14.70
<i>Proteus vulgaris</i>		15.78	25.00	10.71	NR	16.66
<i>Pseudomonas aeruginosa</i>		19.29	25.00	32.14	22.22	23.52

(Figures in parenthesis indicates number of samples)

NR = Not recorded

'n' = Total number of isolates

lower the motility and viability of spermatozoa thereby affecting the fertility of cows (Roberts, 1971). Helt (1961) reported reduced fertility of bull semen contaminated with *Corynebacterium* species. *Escherichia coli* was reported to have spermicidal effects

due to the production of endotoxins (Sakala *et al.*, 1961). The organisms isolated presently are also distributed widely in nature and have been reported to be associated with a wide variety of reproductive disorders in cattle (Saikia *et al.*, 1987).

## Summary

Semen samples (324 samples) from 27 Holstein Friesian bulls were studied for bacterial contamination. Both gram positive and gram negative organisms were isolated from the samples.

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