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Effect of filtration through sephadex and glasswool on the quality and freezability of semen of crossbred bulls

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Nearly 30% of crossbred bulls donate poor quality semen with low freezability rendering them unfit for AI (Pathak 1988, Sahni and Mohan 1988). The objective of the present study was to determine whether glasswool and sephadex-gel column filteration could improve the quality and freezability of semen of triple crossbred bulls.

Ejaculates (40) from 5 triple crossbred bulls (½ HF×¼J× ¼ H) were collected weekly with an artificial vagina and used in a split sample technique. The bulls were maintained under identical conditions at the germplasm centre of the Institute. Slurries of sephadex G-25 (12% w/v) and G-50 (6% w/v) (Pharmacia Co., Upasala, Sweden) and glasswool filter were prepared and semen was filtered according to Vyas *et al.* (1991). The samples were then diluted with tris-citric acid-fructose-yolk-glycerol diluent to give 20-25 millions sperm/0.54 ml French medium straws and frozen in liquid nitrogen after 3 hr equilibration at 5°C. The straws were thawed at 38°C for 30 sec in a water-bath after 12 hr storage in liquid nitrogen and then transferred to an incubator at 37°C. The percentage of motile sperm in initial, pre-freeze and post-thaw (0 hr and 1 hr incubation) samples was assesssed with a phase-contrast microscope (40×) fitted with a biotherm stage. Similarly we determined at initial and post-thaw live/dead and abnormal (head, midpiece, tail and total) spermatozoa. The data were analysed statistically according to Snedecor and Cochran (1967).

Sperm motility

The percentages of motile sperm in initial, pre-freeze and post-thaw (0 and 1 hr of incubation) samples were significantly ($P<0.01$) greater than of control after filtering through sephadex G-25, G-50 and glasswool column (Table 1). Sperm motility in the filterates of glasswool and sephadex G-25 were comparable and both were significantly

Table 1. Mean (±SE) initial, pre-freeze, post-thaw and post-thaw incubation (37°C for 1 hr) motility and percentage of live sperm pre and post-freezing in filtered and unfiltered (control) semen of crossbred bulls

Treatments	Initial motility (%)	Pre-freeze motility (%)	Post-thaw motility (%)	Post-thaw incubation motility (%)	Live sperm (%)	
					Fresh semen	Post-thaw
C	61.38 ±2.36[a]	50.75 ±2.92[a]	25.68 ±2.34[a]	10.75 ±1.64[a]	70.78 ±1.96[a]	32.98 ±2.51[a]
S-25	75.00c ±1.87	66.00c ±2.06	39.78c ±2.38	20.25d ±1.87	83.88c ±3.17	50.22 ±2.44[d]
S-50	71.88 ±2.37[b]	60.62 ±2.52[b]	35.50 ±2.52[b]	15.88 ±1.93[b]	81.10 ±1.59[b]	43.88 ±2.58[b]
G	75.88 ±1.96[c]	65.38 ±1.90[c]	38.62 ±2.43[c]	18.62 ±1.82[c]	83.68 ±1.31[c]	46.98 ±2.46[c]

C, Control (unfiltered); S_{25}, S_{50}, sephadex G_{25}, G_{50}; G, glasswool; means bearing similar superscript between treatments do not differ significantly.

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superior to G-50 at all the stages of their processing. The significant improvement recorded in the initial, prefreeze motility, as well as cryosurvivability of semen filtered through glasswool and sephadex columns supported the findings on bovine (Graham *et al.* 1976, Kumar *et al.* 1989). The filteration of crossbred bull semen was advantageous not only to improve the initial quality but also the freezability and thereby fertility.

Live spermatozoa

In the filterates of sephadex G-25 and glasswool columns the live sperm percentages were similar in initial samples, but differed significantly from one another at the post-thaw stage. The live sperm % in the filterates of sephadex G-50 were, however, significantly lower than the other 2 techniques at both the stages (Table 1). The significant increase in the pre and post-freeze live sperm % over the control in the semen filtered through different columns supports the observation of Paulson and Polakoski (1977), Heuer and Tahir (1982), Kumar *et al.* (1989), and Vyas *et al.* (1991).

Sperm abnormalities

The spermatozoal abnormalities decreased over the control after thawing in the filterates of sephadex G-25, G-50 and glaswool column (Table 2). The mean values for sperm head, midpiece and tail abnormalities were significantly lower ($P<0.01$) in the filterates of all columns at both the stages compared to their control (Table 2). The literature on the effect of filteration of crossbred bull semen on post-thaw abnormalities was not available for comparing the present findings. However, Kumar *et al.* (1989) reported comparable findings in buffalo semen frozen after filteration, through sephadex and glasswool columns.

Immotile/dead spermatozoa retained on sephadex columns may be due to a physico-chemical reaction providing a barrier for immotile sperm cells to agglomarate (Graham *et al.*1976). The separation of spermatozoa was probably the basis of complex and interacting the sperm and the sephadex particles (Landa *et al.* 1980). Lodhi and Crabo (1984), however, found that freeze-killed spermatozoa were trapped to the same extent in the sephadex G-15, polyacrylamide, silica-gel and glasswool columns, indicating that the sperm retention force was not of a chemical nature. Roberts (1972) opined that the spermatozoan's progressive motility was responsible for separation of weak/non-motile sperms through filteration.

It was concluded that the quality of ejaculated semen in respect of sperm motility, live sperm %, abnormal sperm % and freezability of crossbred bulls could be improved significantly by filteration through glasswool and sephadex, particularly G-25. Initially poor quality ejaculates improved markedly following filteration and this technique may be recommended for improving the usage of initially poor ejaculates from valuable crossbred bulls, provided fertility results are

Table 2. Mean (±SE) abnormalities (%) of spermatozoa before and after freezing in filtered and unfiltered semen of crossbred bulls

Treatment	Head abnormalities		Mid-piece abnormalities		Total abnormalities (%)		Total abnormalities	
	Initial	Post-thaw	Initial	Post-thaw	Initial	Post-thaw	Initial	Post-thaw
C	4.60 ±1.11^{b}	6.48 ±1.22^{c}	1.82 ±0.44^{c}	4.18 ±0.67	10.83 ±1.27	12.90 ±1.09^{c}	17.25 ±2.12^{b}	23.56 ±1.92^{b}
S_{25}	2.62 ±0.77^{a}	3.48 ±0.94^{a}	0.70 ±0.27^{a}	1.45 ±0.33^{a}	3.27 ±0.45^{a}	4.25 ±0.40^{a}	6.60 ±1.25^{a}	9.18 ±0.99^{a}
S_{50}	2.50 ±0.75^{a}	4.18 ±0.90^{b}	0.85 ±0.28^{a}	1.68 ±0.36^{ab}	4.45 ±0.48^{a}	5.82 ±0.56^{b}	7.80 ±1.22^{a}	11.68 ±1.16^{a}
G	1.97 ±0.64	4.20 ±0.86^{b}	0.75 ±0.22	1.75 ±0.41^{b}	3.93 ±0.59^{a}	5.77 ±0.52^{b}	6.65 ±1.22^{a}	11.72 ±0.99^{a}

C, Control (unfiltered); S_{25}, S_{50}, sephadex G-25, G-50 columns; G, glasswool column; means bearing superscripts in common do not differ significantly within the column between treatment.

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