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Acrosomal integrity and HOS response at initial, post-thaw and post-refrigeration stages in Sephadex filtered Gir (*Bos indicus*) and Jafarabadi (*Bubalus bubalis*) bulls' semen*

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ABSTRACT

Split-samples of 40 ejaculates of 2 Gir and 2 Jafarabadi bulls (10/bull) were initially filtered through 5 grades of sephadex gel columns (G-25, 50, 75, 100 & 200) to evaluate the relative efficacies of later towards improving the initial quality, freezability and storage ability (at 5°C) of semen in terms of acrosome integrity and HOS test. The mean percentages of sperms with intact acrosome recorded at initial, post-thaw (0 h) and post-refrigeration (48 h) stages were 84.15 ± 0.76, 69.33 ± 1.17 and 73.78 ± 1.00 in the unfiltered semen; 86.50 ± 0.79, 71.53 ± 1.00 and 76.60 ± 1.04 in the filtrates of sephadex G-25; 91.05 ± 0.62, 76.98 ± 0.92 and 81.38 ± 0.90 in the filtrates of G-75; and 94.63 ± 0.64, 82.20 ± 0.86 and 86.35 ± 0.90 in the filtrates of G-200 columns, respectively. The corresponding values for HOS reactive sperm percent were 56.38 ± 1.91, 25.65 ± 1.52 and 32.98 ± 1.56 in the unfiltered semen; 59.90 ± 2.07, 28.50 ± 1.61 and 35.28 ± 1.60 in the filtrates of sephadex G-25; 68.05 ± 2.20, 36.05 ± 1.79 and 43.30 ± 1.88 in G-75; and 75.87 ± 1.96, 42.55 ± 2.02 and 50.18 ± 2.13 in the filtrates of G-200 column. There was significant ($P < 0.01$) and progressive improvement in the percentage of sperms with intact acrosome and HOS response with corresponding decrease in the incidence of swollen, ruffled and denuded acrosome in the filtrates of 5 ascending grades of sephadex. The filtrates of 5 columns registered an increase in intact acrosome and HOS reactive sperms over controls by 2.79 - 12.45 and 6.24 - 34.57% initially, 3.17 - 18.56 and 6.94 - 59.66% at post-thaw stage and 3.82 - 17.04 and 6.97 - 52.15 % at post-refrigeration stage, respectively. The influence of breeds (species), bulls, filtration treatments, breed x stage and breed x bull x stage interactions was highly significant ($P < 0.01$) for all the parameters. Semen quality, as assessed by acrosome integrity and HOS test, was much better in filtrates of higher grades of sephadex (G-75 to G-200) than the lower grades (G-25, G-50) and in Gir than in Jafarabadi bulls and is expected to improve fertility also.

Key words: Acrosomal integrity, HOS test, semen, Gir bulls, Jafarabadi buffalo, Sephadex filtration

Filtration is one of the most practical techniques amongst various physical methods attempted to harvest good quality spermatozoa from the ejaculated semen. The columns of glass-beads (Bangham and Hancock, 1955), glass-fibres (Maki-Laurila and Graham, 1968) and borosilicate glass-wool (Paulson and Polakoski, 1977; Vyas *et al.*, 1992) have been used in past as filtering media to increase the percentages of motile, live and normal spermatozoa in seminal ejaculates, especially of poor quality ones. Filtration of semen through sephadex columns was shown to improve significantly the quality, freezability and/or fertility of bull/buffalo semen (Graham *et al.*, 1976; Graham and Graham, 1990; Chauhan *et al.*, 1993a,b; Goyal *et al.*, 1997). It was hypothesized that filtered rather than unfiltered spermatozoa have stronger plasma membranes that enhance their ability to bear physical stresses during storage, freezing and thawing (Anzar *et al.*, 1997). Better maintenance of sperm motility, morphology, live count, intact acrosome, freezability and sperm penetration into homologus cervical mucus after filtration of buffalo semen through sephadex column have also been reported by Kumar *et al.* (1999). Semen of indigenous Gir and Jafarabadi bulls is observed to be consistently poor in its quality and freezability, inspite of every efforts being made for proper inputs in health care, feeding, and management in stall, at collection and in the laboratory. Hence there is a dire need to improve it by some physical means for which no attempt has been made so far. Therefore, keeping in view this fact and above benefits of sephadex filtration of ejaculated semen, apart from its simplicity and low/negligible cost, the present study was attempted specially to evaluate acrosome morphology and HOS response of spermatozoa of these two breeds at initial, post-thaw and post-refrigeration stages, as they are directly concerned with fertility rates of semen.

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MATERIALS AND METHODS

This study was undertaken at Govt Regional Semen Station, Rajkot during summer (April to June 2001) on 4 sexually mature healthy breeding bulls, 2 of Gir and 2 of Jafarabadi breeds. The bulls were maintained under identical nutritional and managerial conditions and twice a week semen collection schedule. However, the ejaculates (40; 10/bull) obtained at weekly intervals were only utilized for this study. If the initial semen volume was less, a second ejaculate was taken soon to have at least 6 ml total volume.

Preparation of Sephadex gel columns : Slurries of sephadex G-25 (12% w/v), G-50 (6.0% w/v), G-75 (4.2% w/v), G-100 (3.3% w/v) and G-200 (1.8% w/v) were prepared in 3% tri-sodium citrate dihydrate buffer (Graham *et al.*, 1976). They were allowed to swell at least for 4 hrs at room temperature before storage in refrigerator for further use (Heuer and Tahir, 1982). To prepare fresh filters/ columns, 0.6 ml slurries of different grades of sephadex were taken each time by diplip pipette into 5 ml Dispo-Van syringes, which were placed within graduated centrifuge tubes arranged in the test-tube rack (Chauhan *et al.*, 1993a). The rack was then kept in water-bath at 37°C prior to and during filtration. Just before use, each filter was wetted with 2 drops of Tris buffer.

Filtration procedure and evaluation of semen : Initially motile ejaculates were first diluted 1:1 with Tris fructose yolk glycerol diluent. Two ml each of this was then gently put into each Dispo-Van tube containing particular slurry and 2.0 ml was kept as unfiltered control. The control and filtrates of all columns soon after evaluation were further diluted to 1: 15 using TFYG. One aliquot each of these was preserved at 5°C in refrigerator till 48 hrs and another was processed for freezing in liquid nitrogen. Frozen straws (0.5 ml) were thawed in water bath at 37°C in 15 sec. The fresh, post-thawed and 48 hrs refrigerated semen samples (control and filtrates of 5 columns) were evaluated under phase contrast microscope for sperm motility, acrosome integrity (Watson, 1975) and HOS response of sperms (Jayendran *et al.*; 1984) using standard procedures. The acrosomal abnormalities were categorized as swollen, ruffled and denuded, whereas the sperms that reacted with different swelling patterns of tail were graded as type A, B, C and D (Prasad *et al.*, 1999a,b). The data on various traits studied were analysed statistically using multi-factors factorial RBD and critical difference test (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

The breed (species)-wise and overall mean (± SE) percentages of sperms with intact / damaged acrosomes and HOS response of different types observed at initial, post-thaw and post-refrigeration stages of bovine semen processed/ preserved without and with sephadex column filtration are presented in Tables 1-3 and Figure 1. The influence of breeds,

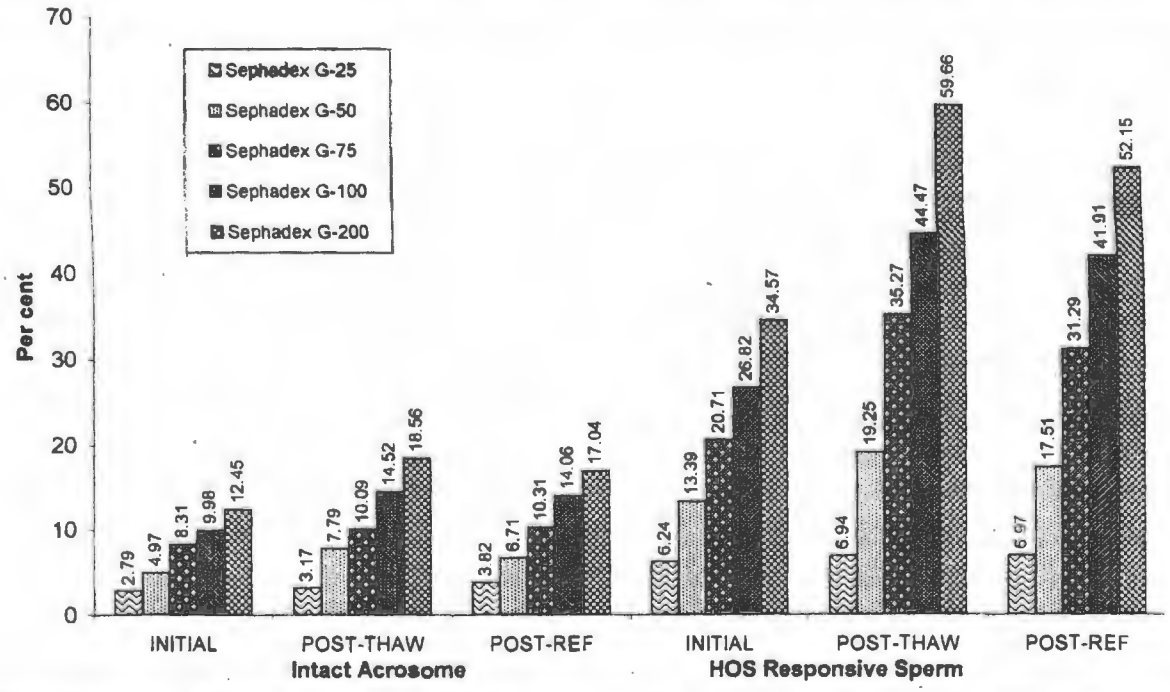

Fig. 1 Per cent increase in intact acrosome and HOS responsive sperm in the sephadex filtered bovine semen over the controls at initial, post-thaw (0 h) and post-refrigeration (48 h) stages

Table 1. Mean (± SE) percentages of intact acrosomes and HOS positive spermatozoa at initial, post-thaw (0 hr) and post-refrigeration (48 hr) in Gir and Jafarabadi bulls' semen processed following filtration through sephadex gel columns

Breed	Treatment	Intact acrosome %			HOS positive spermatozoa %		
		Initial	Post-thaw	Post-refrig.	Initial	Post-thaw	Post-refrig.
Gir	Control	84.80±0.90	70.15±1.61	74.55±1.34	54.35±3.04	26.00±2.48	33.55±2.38
(n=20)	G25	86.90±0.78	71.40±1.26	76.40±1.20	57.85±3.26	29.50±2.44	35.35±2.38
	G50	88.85±0.61	74.65±1.33	79.45±1.28	60.05±3.66	32.10±3.11	38.15±2.89
	G75	91.50±0.50	77.20±1.22	82.30±1.17	64.95±3.93	36.70±2.94	42.95±3.04
	G100	93.30±0.60	80.15±1.30	85.10±1.15	68.50±3.62	40.90±3.01	46.80±3.21
	G200	95.40±0.61	82.65±1.24	87.30±1.09	73.35±3.44	44.25±3.27	49.70±3.60
	Pooled	90.13±0.43**	76.03±0.67	80.85±0.64**	63.16±1.52**	34.91±1.29**	41.08±1.30
Jafari	Control	83.50±1.24	68.50±1.71	73.00±1.50	58.40±2.29	25.30±1.83	32.30±2.08
(n=20)	G25	86.10±1.38	71.65±1.58	76.80±1.72	62.05±2.55	27.50±2.14	35.20±2.21
	G50	87.80±1.23	74.80±1.58	78.00±1.53	67.80±2.11	31.45±1.91	39.35±2.25
	G75	90.60±1.14	76.75±1.38	80.45±1.37	71.85±1.75	35.40±2.10	43.65±2.29
	G100	91.80±0.99	78.65±1.45	83.20±1.45	75.00±1.72	36.10±2.83	46.75±2.44
	G200	93.85±1.12	81.75±1.20	85.40±1.43	78.40±1.79	40.85±2.41	50.65±2.38
	Pooled	88.94±0.57	75.35±0.72	79.48±0.71	68.70±1.04	34.91±1.29**	41.33±1.08
Over- all	Control	84.15±0.76^{a}	69.33±1.17^{a}	73.78±1.00^{a}	56.38±1.91^{a}	25.65±1.52^{a}	32.98±1.56^{a}
(n=40)	G25	86.50±0.79^{b}	71.53±1.00^{b}	76.60±1.04^{b}	59.90±2.07^{ab}	28.50±1.61^{ab}	35.28±1.60^{ab}
	G50	88.33±0.68^{c}	74.73±1.02^{bc}	78.73±0.99^{b}	63.93±2.17^{b}	31.78±1.80^{b}	38.73±1.81^{b}
	G75	91.05±0.62^{d}	76.98±0.92^{c}	81.38±0.90^{c}	68.05±2.20^{c}	36.05±1.79^{c}	43.30±1.88^{c}
	G100	92.55±0.59^{e}	79.40±0.97^{d}	84.15±0.93^{d}	71.50±2.05^{c}	38.50±2.07^{c}	46.80±1.99^{cd}
	G200	94.63±0.64^{f}	82.20±0.86^{e}	86.35±0.90^{d}	75.87±1.96^{d}	42.55±2.02^{d}	50.18±2.13^{d}
	Pooled	89.53±0.36	75.69±0.49	80.16±0.48	65.94±1.12	33.84±0.89	40.61±0.98

** P < 0.01 between breeds, N= number of ejaculates studied. G= Sephadex grade
Overall means bearing different superscripts within the column differ significantly (P < 0.05).

Table 2. Mean(±SE) percentages of sperm with damaged (swollen, ruffled, denuded) acrosomes at initial, post-thaw (0 hr) and post-refrigeration preservation (48 h) of bovine semen processed following filtration through sephadex gel columns

Treatment	Swollen acrosome %			Ruffled acrosome %			Denuded acrosome %		
	Initial	Post-thaw	Post-refrig	Initial	Post-thaw	Post-refrig	Initial	Post-thaw	Post-refrig
Control	2.10±0.23	3.09±0.31	2.93±0.25	3.26±0.31	7.98±0.63	6.90±0.47	10.65±0.71	19.40±1.08	16.65±0.88
G25	1.77±0.21	2.44±0.29	2.37±0.30	2.69±0.29	6.63±0.47	6.03±0.52	09.43±0.69	18.65±0.86	15.28±0.92
G50	1.43±0.21	2.47±0.27	1.99±0.28	1.85±0.20	6.90±0.46	4.95±0.49	08.38±0.59	16.55±0.86	14.35±0.88
G75	1.06±0.13	2.56±0.29	1.77±0.19	1.60±0.21	5.88±0.49	3.83±0.43	06.38±0.51	14.70±0.82	13.00±0.71
G100	0.78±0.14	1.86±0.23	1.54±0.19	1.30±0.16	4.85±0.50	3.47±0.56	05.60±0.58	13.85±0.87	11.20±0.75
G200	0.65±0.11	1.57±0.20	1.49±0.17	0.87±0.14	3.20±0.32	2.43±0.24	03.95±0.53	12.98±0.76	09.75±0.77
Pooled	1.30±0.08	2.33±0.11	2.01±0.10	1.93±0.11	5.91±0.22	4.60±0.21	07.40±0.29	16.02±0.39	13.71±0.37

Table 3: Mean (± SE) percentages of hypo-osmotic swelling reactive (B and C type) spermatozoa at initial, post-thaw (0 hr) and post-refrigeration preservation (48 hr) of bovine semen processed following filtration through sephadex gel columns

Treatment	'B' type swelling			'C' type swelling		
	Initial	Post-thaw	Post-refrig	Initial	Post-thaw	Post-refrig
Control	15.75±1.53	10.08±0.85	12.25±1.12	39.35±1.79^{a}	16.25±1.07^{a}	20.90±1.31^{a}
G25	19.05±1.93	10.23±0.93	11.73±1.07	39.93±2.17^{a}	18.15±1.19^{a}	23.50±1.16^{a}
G50	19.65±1.96	10.45±1.01	11.93±1.05	43.68±1.99^{ab}	21.15±1.34^{b}	26.60±1.30^{b}
G75	19.08±1.60	11.20±0.96	12.25±1.02	46.90±2.60^{b}	24.43±1.40^{c}	30.70±1.48^{c}
G100	19.55±1.96	11.10±0.98	11.30±1.19	50.78±2.37^{c}	26.95±1.70^{cd}	35.05±1.45^{de}
G200	17.98±1.76	09.55±0.89	11.88±1.25	54.98±2.77^{c}	32.00±1.76^{e}	37.90±1.80^{e}
Pooled	18.51±0.73	10.44±0.38	11.89±0.45	45.93±1.00	23.08±0.67	29.11±0.70

Means bearing different superscripts within the column differ significantly ($P < 0.05$).

bulls, stages/periods, filtration treatments and breed x stage and breed x bull x stage interactions was highly significant ($P < 0.01$), while the influence of other interactions was not significant for all the traits (Tables 4-5). The percentage of sperm with intact acrosome and HOS response improved significantly ($P < 0.01$) and progressively with decrease in damaged acrosomes in the filtrates of five ascending grades of sephadex columns i.e. for G-25, G-75 and G-200 over the controls at all stages of evaluation/preservation.

Intact acrosome per cent : The findings on intact acrosomes (Table 1) revealed that there was an improvement over control in the contents of sperms with intact acrosome in the filtrates of 5 ascending grades of sephadex gel columns, viz. G-25, G050, G-75, G-100 and G-200, by 2.79, 4.97, 8.20, 9.98 and 12.45% initially; by 3.17, 7.79, 10.03, 14.52 and 18.56% at post-thaw stage, and by 3.82, 6.71, 10.30, 14.05 and 17.04% at post-refrigeration stage, respectively (Fig. 1). These observations closely agreed with those of Heuer and Tahir (1982), Chauhan *et al.* (1993b), Kanakraj and Easwaran (1994), and Panghal and Tuli (1999). However, Kumar *et al.* (1999) found very marginal improvement in the incidence of intact acrosome following filtration of buffalo semen through sephadex G-15-200 over control, initially (95 vs 97-98%) and after freezing (62 vs 65-66%). Further, the mean percentages of intact acrosome in the control semen of Gir and Jafarabadi bulls (84.80 ± 0.90 and 83.50 ± 1.24) compared well with the reports of Krishna and Rao (1987), Anzar *et al.* (1997) and Prasad *et al.* (1999a). Whereas, Heuer and Tahir (1982) and Sharma *et al.* (1992) recorded lower percentage of intact acrosome in fresh bovine semen. Further the values of intact acrosome found at post-thawed (70.15 ± 1.61 and 68.50 ± 1.71%) or post-refrigeration (74.55 ± 1.34 and 73.00 ± 1.50%) stages of the same samples coincided with the findings of Sharma et al. (1992) and Veeraiah *et al.* (1999), but Chauhan *et al.* (1993b), Panghal and Tuli (1999) and Rasul *et al.* (2000) reported much lower values of intact acrosome (51-62%) in post-thawed semen.

Types of acrosomal damage : The incidence of acrosomal alterations, viz. swollen, ruffled and denuded (Table 2), decreased gradually in the filtrates of different ascending grades of sephadex, i.e. from G-25 to G-200, over the control at all 3 stages of semen processing/ preservation. The incidence of sperm with swollen acrosome declined by 15.71 - 69.05, 19.74 - 49.19 and 19.11 - 49.14 % in different filtrates over the controls at initial, post-thaw and post-refrigeration stages, respectively. The corresponding percent decline in the incidence of ruffled acrosome was 17.48 - 73.31, 16.92 - 59.90 and 12.61 - 64.78 % and that of denuded acrosome 1.46 - 62.91, 3.87 - 33.09 and 8.23 - 41.44 % at 3 stages, respectively. The filtrates of sephadex G-25, G-75 and G-200 columns differed significantly from each other in this regards. No report

Means bearing different superscripts within the column differ significantly ($P < 0.05$).

Table 4: ANOVA showing the effect of breeds (species), bulls, stages, filtration treatments and their interactions on acrosome morphology and HOS positive spermatozoa of Gir and Jafarabadi bulls

Sources of variance		Intact acrosome			HOS positive spermatozoa		
	d.f.	MSS	SEm	CD	MSS	SEm	CD
Ejaculates	9	488.28**	0.60	1.66	4758.65**	1.09	3.02
Breeds (B)	1	210.02**	0.27	0.74	295.16^{NS}	0.49	–
Bulls (BL)	1	199.35**	0.27	0.74	44.49^{NS}	0.49	–
Stages (S)	2	11975.71**	0.33	0.91	68350.11**	0.60	1.65
Treatments (Trt)	5	2413.61**	0.46	1.28	5559.49**	0.84	2.34
Breed x Bull	1	171.42**	0.38	1.04	230.08^{NS}	0.69	–
Breed x Stage	2	7.72^{NS}	0.46	–	980.78**	0.84	2.34
Breed x Treatment	5	10.64^{NS}	0.66	–	25.53^{NS}	1.19	–
Bull x Stage	2	21.00^{NS}	0.46	–	732.11**	0.84	2.34
Bull x Treatment	5	9.19^{NS}	0.66	–	14.23^{NS}	1.19	–
Stage x Treatment	10	12.27^{NS}	0.80	–	12.65^{NS}	1.46	–
B x BL x S	2	213.38**	0.66	1.82	43.17^{NS}	1.19	–
B x BL x Trt	5	9.04^{NS}	0.93	–	48.57^{NS}	1.69	–
BL x S x Trt	10	7.35^{NS}	1.13	–	8.36^{NS}	2.06	–
B x S x Trt	10	2.41^{NS}	1.13	–	16.65^{NS}	2.06	–
B x BL x S x Trt	10	3.91^{NS}	1.60	–	16.82^{NS}	2.92	–
Error	639	25.74	–	–	85.19	–	–

* $P < 0.05$, ** $P < 0.01$, ns non-significant.

Table 5: ANOVA showing the effect of breeds (species), bulls, stages, filtration treatments and their interactions on acrosomal damage (swollen, ruffled and denuded) and hypo-osmotic swelling reactivity (B & C type) of Gir and Jafarabadi bulls' spermatozoa

Sources of variance		Mean Sum of Squares and statistical significance				
	d.f.	Swollen	Ruffled	Denuded	'B' Type	'C' type
Ejaculates	9	25.38**	34.43**	289.11**	960.47**	3100.71**
Breeds (B)	1	10.89**	0.42^{NS}	175.02**	412.38**	1245.84**
Bulls (BL)	1	0.09^{NS}	16.89^{NS}	94.60**	855.21**	418.77**
Stages (S)	2	67.20**	998.71**	4683.58**	4445.59**	33668.82**
Treatments(Trt)	5	34.51**	231.88**	785.96**	40.63^{NS}	4658.18**
Breed x Bull	1	5.25^{NS}	72.02**	54.03^{NS}	762.83**	127.58^{NS}
Breed x Stage	2	6.82*	18.37^{NS}	17.84^{NS}	13.56^{NS}	985.23**
Breed x Trt	5	2.84^{NS}	12.93^{NS}	10.77^{NS}	16.93^{NS}	58.85^{NS}
Bull x Stage	2	1.82^{NS}	1.90^{NS}	56.16^{NS}	307.19**	1706.71**
Bull x Trt	5	0.48^{NS}	6.70^{NS}	11.33^{NS}	21.68^{NS}	38.92^{NS}
Stage x Trt	10	1.12^{NS}	12.97*	4.73^{NS}	33.77^{NS}	17.73^{NS}
B x BL x S	2	13.67**	39.21**	85.53*	149.49^{NS}	406.28**
B x BL x Trt	5	1.74^{NS}	1.95^{NS}	4.35^{NS}	17.77^{NS}	48.35^{NS}
BL x S x Trt	10	1.22^{NS}	1.63^{NS}	3.55^{NS}	12.79^{NS}	23.97^{NS}
B x S x Trt	10	2.61^{NS}	4.38^{NS}	6.84^{NS}	13.59^{NS}	36.77^{NS}
BxBLxSxTrt	10	0.66^{NS}	4.88^{NS}	4.65^{NS}	18.68^{NS}	21.64^{NS}
Error	639	1.75	6.18	21.07	59.22	79.50

* P < 0.05; ** P < 0.01; NS = Non-significant

could be seen in the literature on the effect of sephadex filtration on incidence of different types of acrosomal defects. Although Landa *et al.* (1980) reported that retention of sperm within filters was independent acrosome morphology. Heuer *et al.* (1983), on the contrary, suggested that sperms with membrane defects were trapped in the sephadex filters. Moreover, the pooled means of sperms with swollen, ruffled and denuded acrosome found as 2.10 ± 0.23, 3.26 ± 0.31 and 10.65 ± 0.71%, respectively in the fresh unfiltered semen increased to 3.09 ± 0.31, 7.98 ± 0.63 and 19.40 ± 1.08% in post-thawed semen, and to 2.93 ± 0.25, 6.90 ± 0.47 and 16.65 ± 0.88% in 48 hrs refrigerated semen (Table 2). Sharma *et al.* (1992) and Prasad *et al.* (1999a) also reported more or less similar incidence of such acrosomal changes in the fresh, post-thawed or post-refrigerated bovine semen.

HOS reactive sperm per cent : The percentages of HOS positive sperm recorded at initial, post-thaw and after 48 hrs of refrigeration storage of fresh unfiltered semen (56.38 ± 1.91, 25.65 ± 1.52 and 32.98 ± 1.56) increased progressively and significantly (P < 0.05) in the filtrates of 5 ascending grades of sephadex gel columns, viz. G-25, G-50, G-75, G-100 and G-200, reaching to the values of 75.87 ± 1.96, 42.55 ± 2.02 and 50.18 ± 2.13, respectively, in the later filtrate (Table 1). The percent increase in HOS responsive sperm in the filtrates of these 5 columns over unfiltered control was to the extend of 6.24, 13.39, 20.70, 26.82 and 34.57 at initial stage; 6.24, 19.25, 35.27, 44.47 and 59.66 at post-thaw stage, and 6.97, 17.43, 31.29, 41.90 and 52.15 after 48 hrs of refrigeration storage, respectively (Fig. 1), though the values of control and G-25, G-25 and G-50; and G-75 and G-100 were statistically at par. These findings with respect to effect of filtration on HOS responsive sperms agreed with the report of Anzar *et al.* (1997), who found higher swelling rate of sperm after passing through sephadex ion exchange column than that kept as unfiltered control (19.3 ± 3.8 vs 13.0 ± 1.3%). There were no more reports on the line under study to compare the present findings.

The percentage of HOS positive sperms in Gir bulls' semen was significantly lower than in Jafarabadi bulls at initial stage, but the trend was inversed at post-thaw stage. Moreover, the mean percentages of HOS positive spermatozoa found in the unfiltered semen of both Gir and Jafarabadi bulls

at initial, post-thaw and post-refrigeration were much higher than the values reported by Anzar *et al.* (1997) and Prasad *et al.* (1999b) at initial stage, but compared for post-thaw stage. Brahmkshatri (1995) reported 41 and 53 % HOS responsive sperms in frozen-thawed semen of crossbred and Murrah bulls, respectively. Pramanik *et al.* (1998) reported relatively higher values of HOS positive sperm in fresh (77-80 %) and frozen-thawed (47-60%) buffalo semen, and Rasul *et al.* (2000) for post-thawed cattle sperm (40%).

Curling pattern of HOS responsive spermatozoa : The sperms with B (loose) and C (tight) type curling pattern of its tail showed almost similar trend as that of total HOS responsive sperms in both Gir and Jafari bulls (Table 3). Although, the effect was much pronounced for C than B type curling with respect to filtration treatment at all stages of semen processing/ preservation. The incidence of D type swelling pattern (strongly tight coiling of tail over the head) was very low (only 1-3 %). The influence of all main factors, i.e. breeds, bulls, stages and filtration treatments, and some of the interactions was highly significant ($P < 0.01$) only for the percentage of 'C' type HOS positive spermatozoa (Tables 3, 5). There was significant and gradual enhancement in the percentage of only 'C' type (not B type) HOS responsive sperms in the filtrates of 5 ascending grades of sephadex columns, although the values of control and G-25, and G-100 and G-200 were at par. The 'C' type HOS responsive sperm registered 1.73 - 40.08, 11.69 - 96.92 and 12.44 - 81.34% rise, respectively, over the controls at initial, post-thaw and post-refrigeration stages. The actual data as detailed in the present study for different types of curling pattern of HOS responsive bovine spermatozoa are not available in the literature to compare the present findings. Although, Pramanik et al. (1998) and Prasad *et al.* (1999b) stated that all above types of curling patterns were observed in fresh and frozen-thawed bovine semen.

The present findings on the effect of filtration of bovine fresh semen through different grades of sephadex gel columns revealed that the initial quality of semen was undoubtedly improved especially with higher grades of sephadex. This filtered semen subsequently expressed significant enhancement in its freezability and keeping quality (at 5°C) in terms of not only intact acrosome and HOS responsive sperms, but also in motility, viability and morphology (Rana and Dhami, 2003) at all times, and hence is also expected to enhance fertility rate of that semen. However, to conclude the real impact of sephadex filtration on sperm structure and function, ultra-structural studies and actual fertility trials are warranted.

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