Research Article

Effect of Filtration of Low Grade Ejaculates on Semen Quality Parameters at refrigerated temperature (4-7°C)

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Abstract | To improve the preservability of low grade ejaculates Sephadex and Sephadex ion-exchange, filtration was tried. The study was conducted on split samples of 22 ejaculates of seven Karan Fries bulls maintained at Artificial Breeding Research Centre, NDRI, Karnal, Haryana. The collected ejaculates were extended (1:4) at 30°C and divided in three parts and two parts filtered through sephadex filtration (G-100) (FS), sephadex (G-100) with ion exchanger (FS+IE) and non-filtered serving as control. The semen quality was evaluated at an interval of 0, 24, 48 and 72 hours at refrigerated temperature (4-7°C), respectively. Data was analyzed by analysis of variance technique and comparison between different treatment groups was done by Fisher's Least Significant Difference test. There was significant ($p<0.05$) improvement in motility, hypo osmotic sperm test (HOST), non-eosinophilic count and acrosome integrity upto 48 hrs of preservation at refrigeration temperature, whereas after 72 hrs of refrigeration the parameter showed significant higher values in FS+IE filtration than control. Sperm concentration, tail and total abnormality decreased significantly ($p<0.05$) in FS+ IE and FS filtration as compared to control showed better efficiency of trapping immotile, dead and abnormal spermatozoa. The efficiency of FS+IE columns is further more effective in trapping. Motility and viability after 72hrs of refrigeration showed non-significant difference among the filtered and non-filtered semen samples. In conclusion, sephadex ion exchange filtration is very effective in improving the semen quality as perservability of poor grade.

Keywords | Sephadex, Sephadex with ion exchanger, Motility, Sperm abnormality, Acrosome integrity, Refrigerated temperature, Karan Fries Bull

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INTRODUCTION

India is leading milk producer in the world maintaining 4% growth and targeted 191 Million tonnes milk production and 50% artificial insemination (AI) coverage through production of 140 million frozen semen straws by 2020. To achieve the targeted semen production from superior quality breeding bulls, there is need for harvest of good quality semen without discarding too many poor ejaculates as nearly 20-30% of crossbred bulls donate poor quality semen, rendering them unfit for use in AI (Sahni and Mohan,

1988; Chandrahasan et al., 1986). Therefore, to improve initial quality of poor grade semen of crossbred bulls through technological intervention is always welcome for the wider application to reduce the discard of ejaculate. Generally semen ejaculates with low initial motility is usually discarded due to unacceptable post thaw motility. Besides that, in poor quality semen ROS production is more due to presence of large number of dead and abnormal sperm. Toxic effect of ROS on fertilization potential of companion cells is already reported by various workers (Mac Leod, 1943; Shannon, 1972). ROS acts through lipid peroxidation of carbon chain of unsaturated fatty acid and produce highly cytotoxic lipid hydroperoxides, which decompose to form end product malondialdehyde, which is highly toxic and is responsible for DNA and protein damage finally leading to cell death. ROS production cannot be prevented to full extent but can still be minimized by removing dead and abnormal sperm through bovine serum gradient (White et al., 1984), Percol density gradient (lessley and Garner, 1983), Swim up procedure (Van der van et al., 1988; Mustafa et al., 1998), glass wool filtration (Vyas et al., 1991; Mustafa et al., 1998), glass bead filtration (Daya and Awatkin, 1987), Sephadex filtration (Graham and Graham, 1990; Vyas et al., 1991; Vyas et al., 1992; Anzar and Graham, 1996; Kumar et al., 1999; Vincenti et al., 2002; Ajeet et al., 2003; Maurya and Tuli, 2003; Januskauskas et al., 2005) and sephadex ion- exchanger fitration (Anzar and Graham, 1993; Anzar and Graham, 1995; Anzar and Graham, 1996; Mustafa et al., 1998; Ahmad et al., 2003) and centrifugation techniques (Knop et al., 2005). The sephadex filtration improved the post thaw semen quality and conception rate (Graham and Graham, 1990; Vyas et al., 1992). The trapping mechanism of spermatozoa in filtration columns is not clear, although some speculations are reported by various researchers. In case of sephadex column, sephadex particles either provide a barrier allowing immotile or dead spermatozoa to agglomerate (Graham et al., 1996) or there is a protein present on capacitated spermatozoa, which binds to the sephadex particles (Samper, 1990). In case of Sephadex-ion-exchange filtration, the positively charged dead spermatozoa trapped due to interaction with negatively charged ion-exchanger (Anzar and Graham, 1993). Therefore, the present study was undertaken to evaluate the effect of Sephadex and Sephadex with ion-exchange filtration on various semen quality parameters at refrigerated temperature.

MATERIAL AND METHOD

The present investigation was conducted on Karan Fries (Tharparkar X HF crosses between 50 to 75% exotic inheritance) maintained at Artificial Breeding Complex, National Dairy Research Institute, Karnal, Haryana, India, which is located at latitude 29.43°N and longitude 72.2°E in a semi-arid tract of India.

Semen Collection and Initial Evaluation

Semen was collected in bovine artificial vagina (IMV model-005417) pre-warmed at (42-45°C) with smooth neoprene liner (IMV-005331). On the day of collection, two successive ejaculates were taken with 20 to 30 min gap and each ejaculate was preceded by a period of sexual preparation consisting of at least two false on once a week schedule. Each ejaculate was evaluated for volume and initial motility and the ejaculates having Initial Motility between 55-65% were selected for this study (n=18). Sperm concentration was determined using a haemocytometer (Improved Neubauer, HBG, Germany). The semen was diluted with Tris-citric acid egg yolk glycerol extender. Sephadex and Sephadex Ion-Exchanger Filters were prepared as per the method described by Ahmad et al. (2003) with some modifications. 18 ejaculates were divided into three aliquots, one each for sephadex filtration, sephadex with ion exchanger and control without filtration after extension (1:4) at 30°C. Then the extended semen samples were filtered using the columns.

Semen Analysis

Semen analysis (motility, non-eosinophilic count, HOST and acrosome integrity) were performed for control, FS and FS+IE filtrates at different hours of preservation (0, 24, 48 and 72) at refrigerated temperature (4-7°C). Initial progressive motility rating was scored using 200X magnifications with phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) equipped with a Tokoiheat-heated stage. Percent progressive motility (0–100%) was measured at five representative areas of the slide. The average of the five scores for each category was recorded. If the difference between two consecutive counts exceeds 10 percent, two new counts were performed. Non-eosinophilic (live) spermatozoa (%) were assessed under bright field 100X oil immersion objectives using eosin-nigrosine staining (Blom, 1950; Hancock, 1951). The same slide made for eosin-nigrosine staining was used

for screening morphological abnormalities. About 200 spermatozoa were counted under bright field 100X oil immersion objectives in different fields and percentage of abnormal spermatozoa was calculated by dividing the number of head, mid piece, tail and total abnormalities by the total spermatozoa counted and multiplying the figure by 100. Sperm membrane integrity was assessed using the hypo-osmotic swelling test according to the methods described by Correa and Zavos (1994). Acrosome integrity was carried out by Giemsa staining described by Hancock (1952).

Statistical Analyses

The effect of filtration on different sperm variables at various stages of cryo-preservation was analyzed by analysis of variance technique (Snedecor and Cochran, 1967). Prior to the analysis proportionality data (motility, percent non-eosinophilic count, HOST, acrosome integrity and abnormality data) were transformed using the arcsine transformation [asin (sqrt (percent/100))] (Snedecor and Cochran, 1994) with adjustment to allow for zero values. Comparison between different treatment groups was done by Fisher's Least Significant Difference (LSD) test. The differences at $p \leq 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Table 1-3 depicts the results of filtered semen samples after filtration at different hours of preservation (0, 24, 48 and 72) of refrigerated temperature (4-7°C). The data were analyzed by analysis of variance technique and comparison between different treatment groups was done by Fisher's Least Significant Difference (LSD) test.

Sperm Concentration (X 10^6/ml)

There was significant ($p<0.05$) decrease in mean spermatozoa concentration after sephadex (FS) and sephadex ion-exchange (FS+IE) filtration than the non-filtered samples and among them lowest spermatozoa concentration was observed in FS+IE filtrates.

Semen Quality

Mean individual motility, non eosinophilic count, HOST and acrosome integrity of KF semen was increased significantly immediately after FS and FS+IE filtration and the trend was maintained same up to 48 hours of semen preservation at refrigerated temperature, compared that of non-filtered controls. There was no significant ($p>0.05$) difference between filtered and control sample as well as between FS+IE filtrates and FS filtrates after 72 hrs of refrigeration for motility and HOST. After 72hrs of refrigeration non eosinophilic count and acrosome integrity showed significantly higher values in FS+IE filtration than the control group. The values of individual motility, non eosinophilic count, HOST and acrosome integrity were highest, whereas values of head, midpiece, tail and total abnormalities were lowest in the filtrates of FS+IE followed by FS in all period of refrigeration. There was no significant effect of FS and FS+IE filtration on head and mid-piece abnormalities from 0-72hrs of refrigeration, but the mean tail and total abnormalities were significantly lower ($p<0.01$) in case of FS+IE and FS filtrates as compared to the non-filtered semen samples up to 48hrs of refrigeration.

The present study was conducted to determine the effect of FS (sephadex G-100) and FS+IE (Sephadex-diethyl amino ethane-52 and Sephadex-carboxy methyl-52) filtration on improvement of quality and preservability of poor grade ejaculate of crossbred bull semen to maximize the supply of good quality semen without discarding too many poor quality semen ejaculates. We have selected sephadex G-100 based on the literature regarding improvement of semen quality and higher efficacy of sephadex G-100 (Maurya and Tuli, 2003). The decrease in sperm concentration after FS and FS+IE filtration is the reflection of effective trapping of dead, abnormal and immobile spermatozoa which may be due to either physico-chemical reaction of sephadex particle with immotile or dead spermatozoa leading to agglomeration (Graham et al., 1976) or the protein present on capacitated spermatozoa binds with the sephadex particles (Samper, 1990). On contrary, Anzar and Graham (1993) reported no binding between sephadex particles and sperm cells. In our experiment we found trapped spermatozoa in filtration column. The exact mechanism of trapping spermatozoa in sephadex column is still not clear. Higher efficacy of FS+ IE filtration columns in all semen quality aspect may be due to different trapping mechanism. It is hypothesized that positively charge dead spermatozoa may attached with negatively charge CM-cellulose and get trapped, while motility of negatively charged normal spermatozoa facilitates the passage through DEAE cellulose (An

Table 1: Effect of filtration at room temperature on sperm concentration (x10⁶/ml) of Karan Fries bull semen

Parameter	Treatment					
	C		FS		FS + IE	
	Mean	SE	Mean	SE	Mean	SE
Concentration (x 10⁶/ml)	1003.68^{A}	16.89	725.49^{B}	20.4	649.74^{C}	21.2

Means (±S.E.M.; N=22) with different superscripts within same row differ significantly (^{ABC}P<0.01). (C: Control; FS: Sephadex filter; FS + IE: Sephadex with ion-exchangers)

Table 2: Effect of filtration on semen quality of Karan Fries bull semen at different hours of preservation at refrigerated temperature (4-7°C)

S. No.	Parameters (%)	Hours	Treatment					
			C		FS		FS + IE	
			Mean	SE	Mean	SE	Mean	SE
1	Motility	0	56.65^{A}	0.48	65.27^{B}	0.50	68.59^{C}	0.45
		24	34.82^{a}	0.71	45.39^{b}	0.74	49.30^{c}	0.62
		48	15.90^{A}	0.70	25.87^{B}	0.68	30.63^{C}	0.63
		72	6.38	1.16	7.72	1.08	8.50	1.07
2	Non-eosinophilic count	0	66.43^{a}	0.47	69.25^{b}	0.54	73.77^{c}	0.58
		24	35.09^{A}	0.72	47.90^{B}	0.49	53.03^{C}	0.51
		48	18.53^{A}	0.77	27.60^{B}	0.60	35.91^{C}	0.51
		72	6.11^{A}	0.86	8.48^{AC}	0.95	10.66^{BC}	1.04
3	HOST	0	51.76^{a}	0.71	55.76^{b}	0.75	60.57^{c}	0.74
		24	26.39^{A}	0.95	36.61^{B}	0.84	44.07^{C}	0.88
		48	7.28^{A}	1.22	16.41^{B}	1.08	24.09^{C}	0.94
		72	3.72	1.39	4.73	1.31	5.73	1.36
4	Acrosome integrity	0	68.44^{a}	0.52	71.40^{b}	0.55	75.68^{c}	0.54
		24	45.03^{A}	0.71	52.48^{B}	0.49	57.67^{C}	0.47
		48	25.02^{A}	0.68	32.35^{B}	0.54	38.81^{C}	0.51
		72	14.94^{A}	0.69	16.91^{AC}	0.76	18.28^{BC}	0.69

Means (±S.E.M.; N=22) with different superscripts within same row differ significantly (^{abc}P<0.05, ^{ABC}P<0.01) between treatments. (C: Control; FS: Sephadex filter; FS + IE: Sephadex with ion-exchangers)

zar and Graham, 1993). In both the filtration column spermatozoa normally passes through gravitational and motility force. The results of low sperm concentration after FS and FS+IE filtration of our study is in similar line as reported for cattle (Graham et al., 1976; Anzar and Graham, 1993; Januskauskas et al., 2005), stallion (Jeyendran et al., 1984) and buffalo spermatozoa (Goyal et al., 1996; Panghal et al., 2002; Ahmad et al., 2003).

The results of improvement in motility, viability and intact acrosome as well as effective removal of abnormal spermatozoa post filtration are in consonance with the previous reports of separation using various grades of sephadex in cattle (Graham and Graham, 1990; Vyas et al., 1991; Vyas et al., 1992; Anzar and Graham, 1996; Vincenti et al., 2002; Ajeet et al., 2003; Januskauskas et al., 2005) and buffalo (Heuer et al., 1983; Chauhan et al., 1993; Goyal et al., 1996; Panghal and Tuli, 1999; Kumar et al., 1999; Panghal et al., 2002; Maurya and Tuli, 2003) semen. The motility and viability results of FS+IE filtrates as well as better survivability during liquid storage at refrigeration temperature for longer duration in both the filtration were comparable to that of previous report in Holstein bulls (Anzar and Graham, 1993; Anzar and

Table 3: Effect of filtration on various types of sperm abnormalities (percent) of Karan Fries bull semen at different hours of preservation at refrigerated temperature (4-7°C)

Sno	Sperm abnormalities (%)	Hours	Treatment					
			C		FS		FS + IE	
			Mean	SE	Mean	SE	Mean	SE
1	Head	0	3.43	0.68	3.26	0.73	2.65	0.74
		24	4.14	0.64	3.96	0.72	3.49	0.66
		48	4.73	0.53	4.44	0.59	3.85	0.67
		72	5.09	0.59	4.73	0.59	4.23	0.66
2	Mid-piece	0	2.66	0.60	2.54	0.66	2.05	0.76
		24	3.10	0.59	2.99	0.58	2.30	0.77
		48	3.42	0.42	3.05	0.58	2.55	0.61
		72	3.48	0.53	3.25	0.53	2.95	0.59
3	Tail	0	12.89^{A}	0.59	8.58^{B}	0.68	4.50^{C}	0.79
		24	30.87^{A}	0.62	19.78^{B}	0.68	16.63^{C}	0.41
		48	38.86^{A}	0.46	33.45^{B}	0.51	30.13^{C}	0.60
		72	44.33^{a}	0.53	44.40^{a}	0.38	41.86^{b}	0.35
4	Total	0	19.25^{a}	1.00	14.63^{b}	1.13	9.44^{c}	1.23
		24	38.51^{a}	0.98	27.09^{b}	1.07	22.86^{c}	0.84
		48	47.29^{a}	0.75	41.33^{b}	0.85	36.97^{c}	0.94
		72	53.33^{a}	0.89	52.77^{ac}	0.75	49.51^{bc}	0.67

Means (±S.E.M.; N=22) with different superscripts within same row differ significantly ($^{abc}P<0.05$, $^{ABC}P<0.01$) between treatments. (C: Control; FS: Sephadex filter; FS + IE: Sephadex with ion-exchangers)

Graham, 1995; Anzar and Graham, 1996) and buffalo bulls (Mustafa et al., 1998; Ahmad et al., 2003). HOST and Intact acrosome is highly correlated with fertilization potential of spermatozoa (Saacke and White, 1972; Garner and Hafez, 1987; Osinowo et al., 1982; Saacke et al., 1980). Host reflects the ability of sperm membrane to bear the stress of hypo osmotic solution.

During storage at refrigerated temperature semen quality parameters showed more decreasing trend in control compared to FS and FS+IE may be due to adverse effect of cumulative dead spermatozoa. Dead sperms produce more ROS, which acts through lipid peroxidation of carbon chain of unsaturated fatty acid, which are the important constituent of sperm plasma membrane and thereby results in formation of highly cytotoxic lipid hydroperoxides. These lipid hydroperoxides decompose to form end product malondialdehyde, which is highly toxic and is responsible for DNA and protein damage finally leading to cell death. Adverse effect of ROS become cumulative, therefore fertilization potential of other spermatozoa are compromised. Sperm motility depends on the normal functioning of tail rather than head and mid-piece (Kumar et al, 1989). In the present study non-significant reduction of head and mid-piece abnormality may be due to easier passage of such spermatozoa, where spermatozoa with tail abnormality cannot force themselves to pass through filtration column and get trapped, which resulted in significant decrease of tail as well as total abnormality. The initial and up to 48hrs of liquid storage motility, viability, acrosome integrity and non-eosinophilic count of FS+IE filtrates were significantly better and sperm abnormalities were significantly lower than that of non-filtered and FS filtered semen samples, which indicates greater efficiency of ion-exchangers in removing immotile or dead spermatozoa. Therefore, it can be concluded that fertilization potential of spermatozoa is improved after filtration. Semen quality of filtered and non-filtered spermatozoa after 72hrs of refrigeration did not show any significant differences may be due to adverse effect of dead spermatozoa through ROS production

and mitochondrial aging of sperms leading to production of low quantity of ATP through mitochondrial respiration (Ahmad et al., 2003).The technique can be effective in IVF laboratories for sperm separation.

CONCLUSION

The results of the study depicted that sephadex ion-exchange filtration columns are efficient tool to improve semen quality through removal of immotile, dead and abnormal spermatozoa from low grade ejaculates. The spermatozoa after filtration were able to sustain cryopreservation stress and attain better preservability characteristics. Keeping in view of the ever increasing demands of semen for breed improvement programme to attain targeted milk production, the filtration technique can be used in semen banks as a routine procedure to harvest high quality semen without discarding too many poor ejaculates, provided fertility results are compared and found favourable. Also, this technique can be used after post vaccination latent period till normalcy restored and in seasonal deterioration of semen quality.

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CONFLICT OF INTEREST

The authors have no conflict of Interest.

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