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COMPARATIVE EVALUATION OF DIFFERENT FILTRATION TECHNIQUES FOR IMPROVING THE SEMEN QUALITY OF CROSSBRED BULLS

One hundred and sixty split-samples of 40 ejaculates from five triple crossbred bulls were studied with or without filtration through Sephadex G-25 (12%, 0.6 ml),Sephadex G-50 (6%,0.6ml) and glass wool (100mg, 2 cm) columns in 5 ml glass syringes. The mean initial motility in the unfiltered control and the filtrates of Sephadex G-25, G-50 and glass wool columns were 61.38±2.36, 75.00±1.87, 71.88±2.37 and 75.88±1.96 percent; sperm concentration (x 10 7 /ml) 99.80±6.08 78.02±5.53, 80.05±5.72 and 76.30±5.46, live sperm 70.78±1.98,83.88±3.17, 81.10±1.59 and 83.68±1.31 per cent; sperm head abnormalities 4.80±1.11, 2.62±0.77, 250±0.75 and 1.97+0.64, 0.85±0.28 and 0.75±0.22 per cent; sperm tail abnormalities 10.83±1.27, 3.27±0.45, 4.45±0.48 and 3.93±0.59 per cent, and sperm total abnormalities 17.25±2.12, 6.60±1.25, 7.80±1.22 and 6.65±1.22 per cent, respectively.

Significant differences ($P<0.01$) were observed between bulls, between collections; between treatments (control and 3 filtration techniques) and due to bull x collection interaction for all the parameters; whereas bullx treatment interaction effect was singnificant ($P<0.05$) only for motility and live sperm per cent. In general the filtrates of all the columns were significantly superior to the control. The three filtrates, however, did not differ significantly with regard to sperm abnormalities. the Sephadex G-25 and glass wool were at par and both were superior to G-50 for motility and live sperm percentages. The sperm concentration was significantly reduced after filtration but the loss was compensated by good quality sperm which filtered through. It is concluded that initial quallity and hence usage of poor ejeaculates could be improved effectively by filtration and this may be adopted as a routine procedure for semen of valuable sires.

INTRODUCTION

The low quality semen donated by some 20-30% potential crossbred sires needs to be effectively tackled by suitable laboratory means for its efficient utilization in A.I. work. Different researchers have attempted various physical methods to improve the poor quality semen ejaculates of men and animals. Density gradient centrifugation (Benedict *et al.* 1967; O'Dohnel, 1969), sedimentation (Krzanowki 1970), bovine serum albumin gradient sedimentation (Ericsson *et al.* 1980; David *et al.* 1975), glass beads (Bangham and Hancock 1955) and glass fibre (Maki-Laurilla and Graham, 1958) etc. have been used for sepration of X and Y bearing as well as normal and motile from abnormal and weak/non-motile sperms. Similarly various grades and column heights of Selphadex (Graham *et al.* 1976; Carbo *et al.* 1980; Hiuer *et al.* 1983; Fernandez *et al.* 1985; Chinnaiya *et al.* 1989; Kumar *et al.* 1989) and glass woll (Paulson and Polakoski 1977; Krishanmurthy *et al.* 1983,lodhi and Crabo 1984; Chandrahasan *et al.* 1986; Chinnaiya *et al*, 1989) as filtering media have also been used with promising results in improving the quality of ejaculated semen. Yet the information on Sephadex and glasswool column filtration of crossbred bull semen, whilch is inherently poor, is meagre and hence reported.

MATERIALS AND METHODS.

Forty ejaculates obtained from five triple crossbred bulls under weekly semen collection schedule using artificial vagina were used in a split-sample technique. The bulls were maintained under identical conditions at the Germ Plasm Centre of the Institute, slurries of Sephadex G-25 (20%) and G-50 (6%) (Phermacia Co. Upsala, Sweden) were prepared by allowing them to swell iln 3% Sodium citrate buffer for 4 hrs at 5 °C (Graham *et al.* 1976; Lodhi and Crabo 1984). Filters were prepared in 5 ml glass-syringes by

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placing a thin pad of glass-wool at the botom to support the column. The slurries were loaded into the syringes to the height of 0.6ml mark (1 cm). Glass wool filters were prepared by packaging 100 mg of borosilicate glass wool (E.Merck) into the syringe to a column height of 2 cm (Chandrahasan *et al.* 1986; Kumar *et al.* 1989). All filters were covered with tris buffer just before filtration to make the columns wet and uniformly packed. The syringes holdling the columns were placed vertically over the graduated test-tubes in a stand at 37 °C incubator. Immediately after collection and evaluation for ejaculate volume, density and mass activity 2 ml semen was poured on each of the 3 columns and 1 ml was kept as unfiltered control for each ejaculate. The semen was allowed to drain to the upper level of the filter whilch took nearly 3 min in glass wool and 5-10 min in Sephadex columns. The control whole semen and the filtrates of the 3 columns were then evaluated for sperm concentration by haemocytometer and for the incidence of live/dead and abnormal sperm per cent by eosin-nigrosin stain (Herman and Madden, 1953). The samples were than diluted with tris-fructose yolk glycerol diluent keepilng 40 million sperm per ml in 5 ml test tubes and assessed for the initial progressive sperm motillity. Under a phase contrast microscope (40x) fitted with a biotherm stage (37 °C). The data were analysed statistically using Harvey's fixed model computer programme (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Sperm motility: The mean mass activity (Score 0 to +5) of the semen noted was 2.55±0.14 which varied significantly ($P<0.01$) among the bulls. The

Table 1

Mean ± SE of certain important seminal attributes of cross-breed bulls with or without filtration through different media

Filtration treatment	Initial motility (%)	Sperm Concent. (10/ml)	Live Sperm (%)	Sperm abnormalities(%)			
				Head	Midpiece	Tail	Total
Unfiltered control	61.38^{a} ±2.36	99.80^{c} ±6.08	70.78^{a} ±1.96	4.60^{b} ±1.11	1.82^{b} ±0.44	10.83^{b} 1.27	17.25^{b} 2.12
Sephadex G-25	75.00^{c} ±1.87	78.02^{ab} ±5.53	83.88^{c} ±3.17	2.62^{a} ±0.77	0.70^{a} ±0.27	3.27^{a} ±0.45	6.60^{a} ±1.25
Sephadex G-50	71.88^{b} ±2.37	80.05^{b} ±5.72	81.10^{b} ±1.59	2.50^{a} ±0.75	0.85^{a} ±0.28	4.45^{a} ±0.48	7.80^{a} ±1.22
Glass wool	75.88 ±1.96	76.30 ±5.46	83.68^{c} ±1.31	1.97^{a} ±0.64	0.75^{a} ±0.22	3.93^{a} ±0.59	6.65^{a} ±1.22

Means bearing superscripts in common do not differ significantly between filtration treatments.

Table 2

Analysis of variance (MSS)

Source of variation	d.f.	Initial motility	Sperm concen.	Liv Sperm.	Abnormal Sperm			
					Head	Midpiece	tail	total
Bull	4	426.07**	17419.21**	1659.07**	863.10**	60.65**	41.78*	1480.24**
Collection	7	2984.61**	3167.48**	49.11**	74.26**	7.28NS	26.50NS	138.61**
Treatment	3	117.30**	4792.01**	1529.99**	68.27**	65.68**	462.21**	1368.89**
Bull x Coll.	28	1775.57**	3708.97**	232.10**	54.50**	26.26**	62.84**	158.88**
Bull x Tret.	12	473.72*	65.48NS	33.61*	9.49NS	3.74NS	4.07NS	28.94NS
Coll x Tret.	21	39.51NS	48.42NS	21.96NS	5.62NS	1.68NS	0.68NS	17.59NS
Error	84	26.52	67.15	31.35	5.72	2.84	15.99	24.70

* $P<0.05$; ** $P<0.01$; NS-non-significant.

percentage of initial motility in the control semen averaged 61.38±2.36 (range 25 to 90), which upon filtration through Sephadex G-25, G-50 and glass wool columns improved significantly (by 22.19,17.11 and 22.63 per cent) to 75.00±1.87, 71.88±2.37 and 75.88±1.96 (Table 1).Significant ($P<0.01$) differences in the values were observed between bulls, between collections, between treatments and due to bull x collection and bull x treatment interactions (Table2) indicating that the individual ejaculate as well as bull responded differently to the various filtration media. All the three filtration techniques were found to improve the initial motility over the control. However, the efficiency of Sephadex G-50 was significantly poorer than G-25 or glass wool as regards improving the motility particularly in bulls donating poor quality semen, several researchers using different grades and quantity of Sephadex and glass wool columns have reported varying improvement (10 to 35%) in the percentage of initial progressive motile sperms in the filtrates of human and bovine semen over the control (Graham *et al.* 1976; Poulson and Polakoski 1977; Krishnamurthy *et al.* 1983 ; Heuer *et al.* 1983). The glass wool and Sephadex G-15-200 columns were nearly 100% effective in improving the initial motility of bull and buffalo bull semen (Chandrahasan *et al.* 1986' Chinnaiya *et al.* 1989; Kumar *et al.* 1989). The present findings on crossbred bull semen support their views. According to Roberts (1972) the active movement of sperms was essential for effective passage through such filters.

Live sperm per cent: The mean values for live sperm percentages in the control and the filtrates of Sephdex G-25, G-50 and glass wool column were 70.78±1.96, 83.88±3.17, 81.10 ±1.59 and 83.68±1.31 respectively (Table 1). The values increased by 18.51, 14.58 and 18.22 per cent in the three filtrates over the control. The variations among the bulls, collections, treatments, and bull x collection and bull x treatment interactions were significantly higher than the G-50. These findings are in agreement with those reported in bovines by the above cited workers using different grades and filters. Lodhi and Crabo (1984), however, reported that Sephadex filter was unable to retain dead sperm and those with acrosomal abnormalities. Their observations to some extent corraborate with our findings using sephadex G-50. The exact mechanism of separation of live actively motile sperms by different filters is however, unknown.

Sperm abnormalites: The mean (±SE) values and the analysis of variance for the abnormalities of sperm head, midpiece, tail and total are presented in Tables 1 and 2. Following filtration of split-samples through Sephadex G-25, G-50 and glass wool colulmns, the incidence of sperm abnormalities decreased by 43.04, 45.65 and 57.17 per cent in the head region; 24.35,21.09 and 23.26 per cent in the midpiece; 61.81, 58.91 and 63.71 per cent in the tail region, and 61.74, 54.78 and 61.45 per cent in total sperms, respectively, over the control. The influence of bulls, collections, filtration treatments and bullxcollection interaction was significant. Thus an average of 45 to 65% reduction in the sperm abnormalities of head, tail and total could be achieved by various filtration techniques over the control. These findings corraborated well with the earlier reports on cattle and buffalo semen (Graham *et al.* 1976; Hener *et al.* 1983; Lodhi and Crabo 1984; Chandrahasan *et al.* 1986; Chinnaiya *et al.* 1989; Kumar *et al.* 1989).

It was concluded that the quality of ejaculated semen In respect of sperm motility, live sperm motility, live sperm per cent of crossbred bulls could be improved siginificantly following the use of glass wool and Sephadex, particularly G-25, column filtration over the unfiltered whole semen. Further the initially poor quality ejaculates improved markedly than the good ones following filtration and hence this technique is recommended for routine use in the semen banks for improving the quality and usage of initially poor ejaculates from valuable crossbred bulls.

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