The Indian Journal of Animal Reproduction: 23(2): 120 - 122; December 2002

Improvement of buffalo bull semen quality through sephadex filtration and subsequent assessment through polyacrylamide gel column migration

SATWINDER SINGH[1] , G. R. PANGAONKAR[2] , K. C. CHOUDHARY[3] AND H. K. VERMA[4]

Department of Animal Reproduction, Gynaecology & Obstetrics, College of Veterinary Sciences, Punjab Agricultral University, Ludhiana - 141 001

Received : February 17, 2001
Accepted : February 7, 2002

ABSTRACT

To study the effect of sephadex (G 15- 120) filtration in improving the buffalo bull semen quality, 20 ejaculates from four bulls exhibiting poor freezability were taken. Sephadex filtered Tris-yolk extended semen exhibited significant ($P < 0.05$) improvement in sperm motility (63.75 ± 0.99 vs 71 .75 ± 0. 81 per cent), live sperm count (75.15 ± 0.65 vs 83.00 ± 0.70 percent) and spermatozoal penetrability through PAG column (20.35 ± 0.26 vs 22.43 ± 0.32 mm/20 min.) as compared to unfiltered semen. Subsequently sephadex filtration improved the freezability of semen by increasing ($p < 0.05$) its post thaw motility (29.50 ± 0.85 to 42.75 ± 1.02 percent), livability (41.60 ± 0.88 to 52.90 ± 1.30 percent) and percent spermatozoal penetrability through polyacrylamide gel column (17.71 ± 0.07 to 19.41 ± 0.16 mm/20 min.).

Key words : Buffalo semen quality, Sephadex filtration, freezing of semen, sperm motility

Sometimes, semen samples of bulls carry morphologically normal spermatozoa intermixed with abnormal, non-motile sperms and other cellular and non-cellular debris. Such samples have poor keeping quality and freezability, results in lowering fertility. Fractionation of morphologically normal motile sperms from abnormal sperms and cellular debris is likely to improve the quality of semen. Sephadex column filtration technique has been used by various workers (Heuer *et al.*, 1983, Graham and Graham. 1990 and Cisale, 1998) to improve the initial quality of semen. The present study was designed to find out the effect of sephadex column (G-15-120) filtration to improve the quality and freezability of buffalo semen and subsequent assessment through polyacrylamide gel column migration.

MATERIALS AND METHODS

The study was carried out in four buffalo bulls exhibiting poor freezable ejaculates viz. post thaw motility < 40 percent. A total of 20 ejaculates, five from each bull were collected at regular intervals. The semen samples after initial evaluation were extended in Tris egg yolk extender. A 20 percent (W/V) slurry of sephadex (G 15-120) was prepared in 3 percent sodium citrate and the column was prepared in a sterile syring (Kumar 1989). The extended semen was then divided in to Aliquot A, unfiltered and Aliquot B filtered through sephadex column. Both unfiltered and filtered extended semen was thereafter processed for Cryopreservation at -196°C. The semen samples were (both unfiltered and filtered) examined for sperm motility, normal sperm count and live sperm count. The synthetic migration media viz. two percent polyacrylamide gel was prepared (Lorton *et al.*, 1981) and the extended semen samples (unfiltered and filtered) were assessed for polyacrylamide gel column sperm penetration before and after cryopreservation.

RESULTS AND DISCUSSION

The mean (±SE) values of various seminal Characteristics and spermatozoal penetrability through polyacrylamide gel column in extended and post thawed semen before and after sephadex column filtration are presented in Table 1. Sephadex filtered Tris egg yolk extended semen samples exhibited significant ($p<0.05$) improvement in spermatozoan motility (63.75 ± 0.99

[1]Veterinary Officer, Punjab State.
Corresponding author - [2]Professor, Deptt. of Animal Reproduction. Gynaecology & Obstetrics. PAU, Ludhiana. [3]Professor & Head, Deptt. of Vety. Biochemistry, PAU. Ludhiana [4] Assistant Professor, Deptt. of Vety. & A.H. Extension. PAU, Ludhiana.

Table 1. Seminal characteristics of poor freezable ejaculates and the SPD value at extended and post thawed stage in unfiltered and sephadex column filtered buffalo semen.

Parameters	Unfiltered semen (n=20)	Sephadex column filtered semen (n=20)	t-value
Extended semen			
Sperm motility (%)	63.75 ± 0.99	71.75 ± 0.81	6.91*
Live Sperm Count (%)	75.15 ± 0.65	83.00 ± 0.70	8.01*
Normal Sperm Count (%)	81.70 ± 0.64	89.95 ± 0.31	20.33*
SPD value (mm/20 min)	20.35 ± 0.26	22.43 ± 0.32	5.44*
Post thawed semen			
Sperm motility (%)	29.50 ± 0.85	42.75 ± 1.02	11.46*
Live sperm count (%)	41.60 ± 0.88	52.90 ± 1.30	9.74*
Normal Sperm Count (%)	74.35 ± 1.18	84.90 ± 0.48	19.73*
SPD value (mm/20 min)	17.71 ± 0.07	19.41 ± 0.16	13.81*

* ($P < 0.05$)

vs 71.75 ± 0.81 percent), live sperm count (75.15 ± 0.65 vs 83.00 ± 0.70 percent), normal sperm count (81.70 ± 0.64 vs 89.95 ± 0.31 percent) and sperm penetration distance (SPD) through polyacrylamide gel column (20.35 ± 0.26 mm vs 22.42 ± 0.32 mm) as compared to unfiltered semen. Similar results have been observed by Goyal *et al.* (1996), Kanakraj *et al.* (1996) and Kumar (1996). The improvement in seminal characteristics might be due to the retention of dead and immotile spermatozoa in the sephadex column since these dead and immotile cells aglomerate with sephadex particles on account of increased stickiness which developed after their death. Beker and Degen (1972), Graham *et al.* (1976) and Roberts (1972) noticed that motile spermatozoa kept in column orient themselves downwards due to gravitational force, while dead spermatozoa float at the top since they loose the power of orientation. The active movement of spermatozoa is essential for active passage through sephadex column.

The post thaw motility (29.50 ± 0.85 vs 42.75 ± 1.02 percent), live sperm count (41.60 ± 0.88 vs 52.90 ± 1.30 percent), normal sperm count (74.35 ± 1.18 vs 84.90 ± 0.48 percent) of sephadex filtered semen significantly ($P<0.05$) improved over unfiltered extended semen. The overall sperm penetration distance (SPD) in polyacrylamide gel column of post thawed unfiltered and post thawed sephadex column filtered semen was recorded as 17.71±0.07 and 19.41±0.10 mm/20 min respectively. The differences between the values were significant ($P<0.05$). Similar results have been reported by Kumar *et al.* (1996). Further, the significant increase in SPD value might be due to more number of progressive motile and morphologically normal spermatozoa in the filtered extended semen. A significant correlation has been observed between sperm motility and sperm penetration through polyacrylamide gel by Eggert-Kruse *et al.* (1993) and Kaushal (1998). It was concluded that the poor freezable ejaculates could be successfully frozen after filtration through sephadex column.

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