Quality and Fertility of Post Thaw Sephadex Filtered Bull Semen

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ABSTRACT : The present investigation was carried out to assess the effect of Sephadex (G-15) filtration on the post thaw bull semen quality and conception rate. Post thaw unfiltered (control) and Sephadex filtered semen from four healthy bulls (three cross bred and one pure bred Holstein Friesian) were subjected to microscopic examination viz. sperm concentration, individual motility, live sperm count and sperm morphology. Sixty-two healthy, normal cycling crossbred cows were inseminated with post thaw unfiltered (n=32) and filtered semen (n=30). Sephadex filtration of post thaw semen significantly ($p<0.05$) decreased total sperm concentration and sperm with abnormal head, mid piece and tail. The overall average total sperm concentration, head and tail defects in filtered semen decreased significantly (53.4, 1.2 and 6.4 million) than in the unfiltered semen (80.4, 2.4 and 15.7 million, respectively). However, after filtration significant ($p<0.05$) increase in overall average motile and live sperm concentration were observed (38.8 and 38.0) as compared to unfiltered semen (29.2 and 32.0 million, respectively). The overall conception rate recorded was 21.9% with post thaw unfiltered semen and 56.7% with filtered semen. It was concluded that Sephadex filtration of post thaw semen improved its quality and conception rate.
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Key Words : Bull, Semen, Sephadex Filtration, Fertility

INTRODUCTION

Overall conception rate to artificial insemination in cattle varies from 20 to 65 percent in different countries (Rankin et al., 1992; Fonseca et al., 1995; Kommisrud, 1996; Markvichitr et al., 1997; Maurya, 2000). Apart from various infectious, hormonal and anatomical factors, poor post thaw motility affect conception rate in bovine (Krishnamurty et al., 1974; De et al., 1982). Individual sperm motility and live sperm count decrease significantly following freezing and thawing of bull semen (Sahu and Pandit, 1997; Singh et al., 1997). Dead sperm releases hydrogen peroxide (Shannon and Curson, 1972) that has lethal effect on sperm (Alvarez et al., 1987). Thus increased proportion of dead and abnormal sperm has negative effect on fertility (Saacke, 1970). Removal of dead sperm in the thawed semen might therefore help improving its fertility.

Sephadex filtration (Graham et al., 1976) is one of the physical methods, which have been tried to separate normal, motile sperm from abnormal and dead sperm in human and animal's fresh semen in order to improve semen quality (Maki-Laurila and Graham, 1968; Mc Grath et al., 1977; Paulson and Polakoski, 1977; Luderer et al., 1982; Wall et al., 1984; Estiennen et al., 1988; Agarwal et al., 1991). Filtration of fresh and/or diluted bull semen through Sephadex column increased the proportion of normal motile and live sperm up to 68 percent (Fayemi et al., 1979; Graham and Graham, 1990; Kanakraj et al., 1996). However, information about the effect of Sephadex filtration on the quality and fertility of thawed semen is meager. The present study therefore, was planned to filter the post thaw semen through Sephadex (G-15) column and to know its effect on quality and conception rate following artificial insemination.

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MATERIALS AND METHODS

Frozen semen from four healthy bulls [Three cross bred (HF×Sahiwal) and one pure bred Holstein Fresian] maintained at dairy farm, Punjab Agricultural University, Ludhiana was used in this study. Bulls were fed with seasonal green fodder, wheat straw and concentrate. Fifty medium sized french straws (length 135 mm, diameter 2.8 mm and volume 0.5 ml) with frozen semen from each bull were used for the studies either in the unfiltered (control group) or after Sephadex filtration (treatment group). Semen samples from each bull were subjected to post thaw microscopic examination viz. total sperm count, individual sperm motility, live sperm count, sperm abnormalities and fertility trial.

Preparation of Sephadex column

Sephadex G-15 (particle size 40-120 microns, Pharmacia Fine Chemicals, Uppsala, Sweden) columns were prepared as per the methods described by Graham et al. (1976). Sephadex slurry, 20% (W/V) was prepared in 3% sodium citrate solution (pH 6.8) and allowed to swell in refrigerator for at least three hours. Sterilized glass wool (100 mg) was placed at the bottom of a 5 ml sterilized glass syringes and Sephadex slurry was added to each to prepare 0.6 ml columns. The columns were stored at 5°C till use.

Table 1. Spermiogram of unfiltered (UF) and post thaw Sephadex filtered (F) bull semen

Bull No.	n	Total sperm (million/ml)		Motile sperm (million/ml)		Live sperm (million/ml)		Head defect (million/ml)		Mid-piece defect (million/ml)		Tail defect (million/ml)	
		UF	F	UF	F	UF	F	UF	F	UF	F	UF	F
HHS 973	8	77.5±1.29	53.5±1.91*	29.0±2.30	39.4±1.78	31.7±1.64	38.5±1.95	2.6±0.44	1.3±0.97	2.5±0.56	1.7±0.88	13.7±1.04	5.3±0.89*
HF 804	10	81.6±2.06	53.8±1.41*	27.4±1.95	37.6±2.11	29.5±1.64	37.8±2.18	2.1±0.47	1.2±0.80	2.2±0.48	1.4±0.89	16.9±0.68	7.1±1.16*
HF 1013	9	78.9±1.74	52.0±1.73*	27.9±1.83	38.0±1.82	30.7±1.81	35.9±2.50	2.3±0.57	1.3±0.80	2.4±0.42	1.4±0.89	14.9±1.24	5.9±1.22*
AHF 113	7	84.0±1.85	54.6±1.66*	33.8±2.69	40.6±2.32	37.7±1.78	40.4±1.94	2.6±0.41	1.0±0.62	2.2±0.48	0.8±0.69	17.3±1.02	7.2±2.67
Overall average	34	80.4±0.96	53.4±1.70*	29.2±1.11	38.8±2.03*	32.0±0.96	38.0±2.20*	2.4±0.23	1.2±0.82*	2.3±0.23	1.3±0.88	15.7±0.54	6.4±1.18*

* Values differ significantly ($p<0.05$) from the corresponding unfiltered semen.

Filtration of semen

Semen was filtered for use in AI as and when the cows were in estrus. The rack with syringe containing Sephadex column was placed in an incubator at 37°C for 30 min. Prior to filtration the column was made wet with 3 to 4 drops of 3% (W/V) sodium citrate solution. At any one time, two straws of frozen semen from a bull were thawed at 37°C for 30 sec and gently placed over the Sephadex column. Filtered semen was collected in a 2 ml Eppendorf tube. The filtration process was completed within 2 to 3 min at 37°C protected from bright light and draught.

Spermiogram

Total sperm concentration (million/ml) was measured by neu-baur chamber method. Individual sperm motility was estimated for both unfiltered and filtered semen by taking a drop of semen on clean, grease free, pre warmed glass slide, covered with a cover slip and placed on biotherm at 37°C. At least 5 to 6 fields were observed under final magnification of ×400 (×40 objective, ×10 eyepiece) and only progressively motile sperm were considered to estimate motility as 0 to 100%. Live sperm count was estimated by differential staining technique using Eosine-nigrosine stain (Blom, 1977). A total of 200 spermatozoa were counted in 5 to 6 different fields under oil immersion lens and percent live sperm was calculated. Abnormal sperm count was studied from the thin smear stained with Rose Bengal stain (Sharma, 1987). Total number of motile, live and abnormal sperm in unfiltered and filtered semen was calculated by multiplying % motile, % live and % abnormal sperm with total number of sperm.

Fertility trial

Fertility trial was conducted on Sixty-two healthy, normal cycling crossbred (HF×Sahiwal) cows. Estrus detection was performed by vasectomized bulls and then confirmed by per-rectal palpation of genitalia. Cows were divided randomly into two groups (Group-1; n=32) and (Group-2; n=30). Cows in Group-1 were inseminated with one straw each having post thaw unfiltered semen and those in Group-2 were inseminated with post thaw Sephadex filtered semen. Each cow was inseminated twice at an interval of 12 h during standing estrus.

Insemination technique

Fresh empty straws (cut from open end by one cm.) were sterilized by exposing them under UV light for 4 h. Two straws of thawed semen were pooled in a 2 ml Eppendorf tube. Semen (0.5 ml) was aspirated into an empty straw with the help of an adaptor and a tuberculin syringe. AI was performed in Group-1 cows using this straw within 5 min after pooling. Same protocol was followed after filtration for insemination in Group-2 cows.

Pregnancy diagnosis

The inseminated cows were kept under observation for return of heat. Conception was confirmed in non-return cows by per-rectal palpation of genitalia after two month of AI. Conception rate (CR) was calculated as per formula described by Nair (1975)

$$CR=\frac{\text{Number of animals pregnant}}{\text{Total number of animals inseminated}}\times 100$$

Statistical analysis

Results obtained were statistically analyzed as and where required using student's t test.

RESULTS

Spermiogram

The details of total sperm concentration, concentrations of motile, live and abnormal sperm in post thaw bull semen with or without filtration are presented in Table 1. Sephadex filtration of post thaw semen significantly ($p<0.05$) decreased total sperm concentration and sperm with abnormal head, mid piece and sperm tail. The overall average total sperm concentration, head and tail defects in filtered semen decreased significantly (53.4, 1.2 and 6.4 million) than in the unfiltered semen (80.4, 2.4 and 15.7 million, respectively). After filtration, concentration of motile and live sperm increased non-significantly in each bull. However, significant ($p<0.05$) increase in overall average motile and live sperm concentration was observed after filtration (38.8 and 38.0) as compared to unfiltered semen (29.2 and 32.0 million, respectively).

Table 2. Conception rate in cows inseminated with post thaw unfiltered and Sephadex filtered bull semen

Bull No.	Unfiltered semen			Filtered semen		
	No. of cows inseminated	Pregnant	Percent conception	No. of cows inseminated	Pregnant	Percent conception
HHS 973	10	1	10.0	9	4	44.4*
1F 804	6	2	33.3	10	5	50.0*
1F 1013	10	2	20.0	6	4	66.7*
AHF 113	6	2	33.3	5	4	80.0*
Overall average	32	7	21.9	30	17	56.7*

* Indicates values significantly ($p<0.05$) higher than in the unfiltered semen.

Fertility trial

Observations on fertility trial using post thaw unfiltered or filtered semen is given in Table 2. Conception rate (CR) in cows inseminated with unfiltered semen varied from 10.0 to 33.3%. The cows inseminated with filtered semen had CR between 44.4 and 80.0%. The overall average CR with post thaw filtered semen was significantly ($p<0.05$) higher (56.7%) than with the unfiltered semen (21.9%).

DISCUSSION

Spermiogram

All the parameters of spermiogram varied within normal range. Such report on post thaw Sephadex filtered semen is not available for comparison. However, similar decrease in total sperm concentration after Sephadex filtration of extended bull semen were also reported by Kanakraj et al., 1996 (209.93 to 95.66 million/ml), Chauhan et al., 1993 (1.114 to 0.962 billion/ml). Similar increases in motile sperm proportion were also reported by Graham et al., 1976 (up to 90%), Fayemi et al., 1979 (45 to 68%) and Graham and Graham, 1990 (31 to 52%). Filtration increased live sperm proportion from 54.95 to 62.53% (Kanakraj and Easwaran, 1994), 56.94 to 80.94% (Kumar et al., 1992), and 70.78 to 83.88% (Vyas et al., 1992). The increase in proportion of motile and live sperm in filtered semen is considered to be due to retention of dead, abnormal or immotile spermatozoa in the Sephadex column (Graham et al., 1976). The filtration of sperm was probably on the basis of complex and interacting properties of sperm plasma membrane, the medium suspending the sperm and Sephadex particles (Linda et al., 1980). Lodhi and Crabo (1984) hypothesized that leakage of certain macromolecules from dead and abnormal spermatozoa bind to the Sephadex beads leading to their retention. Dead spermatozoa adhere to Sephadex beads due to certain physico-chemical reaction (Graham et al., 1976) or increased stickiness of the spermatozoa after death (Baker and Degen, 1972) might be responsible for their retention.

Sperm abnormalities were within normal range. Filtration significantly increased the proportion of normal sperm. Data on sperm abnormalities after Sephadex filtration of post thaw semen is not available. However, filtration of extended semen decreased sperm with abnormal head (4.60 to 2.62%) mid piece (1.82 to 0.70%) and tail (10.83 to 3.27%) Vyas et al., 1992. Graham and Graham (1990) reported decrease in sperm head and mid piece abnormalities after filtration from 14.3 to 11.6% and from 4.1 to 3.2%, respectively. Sperm pass through Sephadex column due to its own motility (Roberts, 1972). Tail is responsible for sperm motility (Tilney et al., 1973). So, non-significant decrease in head and mid piece abnormalities after filtration may be due to passing of spermatozoa having these abnormalities through Sephadex column.

Fertility trial

CR differed widely following AI with unfiltered semen as well as filtered semen from different bulls. Kanakraj et al. (1996) reported 37.61 percent CR with unfiltered semen and 61.5% with pre-freeze Sephadex filtered semen. Graham and Graham (1990) also observed increased non-return rates (61 vs. 67%) for the low fertility bulls. However, no data on CR is available with post thaw filtered semen for comparison. Almost 80-90% A.I. is done with frozen thawed semen (Kommisrud, 1996). Freezing and thawing increase the sperm associated abnormalities (Singh et al., 1997) and decreases CR following AI (42%) as compared to fresh semen (62%; Howlader et al., 1997). Damaged/dead sperm plasma membrane stimulates reactive oxygen species production (Aitken and Clarkson, 1989), which damages the healthy sperm membrane affecting its fluidity and DNA strand configuration (Kodoma et al., 1997). Moreover, toxic effects are enhanced in the presence of egg yolk (Romanoff and Romanoff, 1949), whose concentration might have decreased after filtration reducing toxin production. Sofikitis et al. (1992) reported that the filtration of semen yielded sperm with higher acrosin activity. So, the increased CR using post thaw Sephadex filtered semen may be due to increased proportion of motile, live sperm having higher acrosin content thereby providing more competent spermatozoa for fertilization. Present study indicates that insemination with post thaw filtered semen increase the conception rate in cattle.

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