

Semen Quality of Holstein and Buffalo Bulls after Filtration using Sephadex Column

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Abstract

To evaluate the effect of sephadex column filtration technique on semen quality of five Holstein bulls and five Egyptian buffalo bulls. Semen was collected biweekly from each eight weeks. Immediately after collection, semen was extended (37°C) and filtered using sephadex column-filtration technique. Semen was evaluated for physical semen characteristics including, percentages of sperm motility, live sperm and sperm abnormality as well as sperm cell concentration pre-and post-filtration. Results show that among all physical semen characteristics, only ejaculate semen volume was significantly ($P < 0.001$) higher in Holstein than buffalo bulls, but motility, livability, abnormality, sperm concentration and sperm with intact acrosome did not differ between both species. As a result of filtration, sperm motility and livability increased ($P < 0.05$) by 16.4 and 11.8% in Holstein and by 16.9 and 10.1% in buffalo semen, respectively. Sperm abnormality and concentration reduced ($P < 0.05$) by 2.6 and 3.3% in Holstein and by 2.4 and 3.5% in buffalo semen, respectively. Improvements of live sperm and the reduction in sperm concentration (proportional to the pre-filtration value) were better ($P < 0.05$) in Holstein than buffalo semen (15.5% and 52.4% vs. 13.2 and -49.3%, respectively). Improvement of motility and abnormality did not differ in Holstein (25.4 and 57.8%) and buffalo semen (26.6 and 54.5%), respectively. The present results indicate that using sephadex column filter technique has beneficial effects on improving quality of spermatozoa in both species.

Keywords: Holstein, buffaloes, semen, sephadex column filtration technique.

Introduction

Mammalian spermatozoa are characterized by marked morphological heterogeneity in an ejaculate. Dead and abnormal spermatozoa have toxic (Shannon and Curson, 1972) and lytic (Lindemann *et al.*, 1982) effects on companion cells in semen, and consequently reduce fertility (Saacke and White, 1972).

In natural mating, cervical mucus differentially selects motile spermatozoa and acts as a barrier to immotile ones (Saacke, 1984). This cervical selection is by-passed in artificial insemination. Methods of separation of motile, normal, or live from immotile, abnormal, or dead ones received little attention in the earlier reports.

The first successful separation of motile from immotile spermatozoa was performed by passing diluted semen through a layer of small glass beads (Bangham and Hancock, 1955), glass wool (Maki-Laurila and Graham

(1968), pyrex beads (McGrath *et al.*, 1977), Newtonian gel (Luderer *et al.*, 1982), Percoll gradients (Lessley and Garner, 1983), bovine serum albumin gradients (White *et al.*, 1984 and Zavos, 1985), the swim-up method (Parrish and Foote, 1987) and sephadex (Maki-Laurila and Graham, 1968 and Graham *et al.*, 1976) filtration method.

A significant increase in the percentage of sperm motility and live spermatozoa of bull (Maki-Laurila and Graham, 1968 and Graham *et al.*, 1976) and goat (El-Saidy, 2000) after filtration through sephadex.

The previous reports indicated species differences in response of immotile, dead, abnormal spermatozoa to filter through sephadex column.

Therefore, the current study aims to compare the response of spermatozoa of Holstein and buffalo bulls throughout sephadex column filtration technique.

Materials and Medhods

This study took place at the International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture.

Animals

Five Holstein bulls (7-10 years) and five buffalo bulls (9-11 years) raised at the ILMTC were used in this study. All bulls were highly fertile and free of any diseases with healthy appearance.

Collection and evaluation of semen

The semen used in this study was collected from the experimental bulls as a common practice in ILMTC. The semen was collected twice a week from each bull using the conventional artificial vaginal method, where one ejaculate from each bull per week was taken immediately to the laboratory and was kept in water bath at 37°C for performing evaluation tests. Semen ejaculates were collected before feeding at 8.00 a.m. A bull was used as teaser animal for sexual preparation. The collection of semen for this study was undertaken during the period from June to October, 2000.

Evaluation of semen

A total of eight ejaculates were conventionally evaluated in the semen of each of the Holstein and buffalo bulls (i.e. total of 40 ejaculates for each species). Immediately after semen collection, each ejaculate was evaluated according to the conventional methods for ejaculate volume, sperm cell concentration, and percentages of sperm motility, live sperm, and sperm abnormalities (Blom, 1983). Percentage of sperms with intact acrosome was also examined using Giemsa stain (Sigma Chemical Co. St. Louis, Mo.).

Sephadex column-filtration test

Preparation of extenders

Portions of Holstein ejaculates were extended at a rate of 1:20 in heated (37°C) Na-citrate and egg yolk extender, while portions of buffalo ejaculates were extended at the same rate in heated (37°C) Tris extender.

The stock solution of the extender of Holstein semen was prepared with Na-citrate (2.9%) egg yolk (20%), glycerol (8%), streptomycin (0.005 g), lincomycin (0.25%) and distilled water (3 times) to 100 ml.

The extender of buffalo bulls (Tris) was prepared with Tris (0.05 g), citric acid (3.35 g), streptomycin (0.5 g), lincomycin (0.01 g) and distilled water (100 ml), then 16 ml egg yolk, 12 ml glycerol and 72 ml distilled water were added to obtained 200 ml of the Tris extender. Both extender and ejaculates were kept and mixed at 37°C.

Preparation of slurries

A total of 20 g sephadex G-25-150 (Sigma Chemical Co. St. Louis, Mo.) was added to 100 ml sodium citrate (3%) (Flushing fluid) for test analysis. Sephadex particles were mixed and allowed to swell for a minimum of 3 h at room temperature to obtain 20% (W/V) sephadex slurries.

Preparation of Sephadex filters

The filtration column was prepared in a 10 ml disposal plastic syringe. A small amount of glass wool was compressed with the plunger to the bottom of the barrel (1ml mark) to prevent the loss of sephadex. An amount of one ml of sephadex gel was gently layered over the glass wool and allowed to settle for 1-2 minutes.

Filtration of extended semen

Extended semen (20 ml) was gently placed on the column using pipette through 3-4 doses at 37°C. It was allowed to drain from the column to complete filtration for about 10-15 minutes for each test. The filtrate was collected in 50 ml volumetric flasks.

Semen evaluation related assays

With each filtered and non-filtered sample, percentages of live and abnormal spermatozoa were determined as described in evaluation of raw semen. However, percentage of individual sperm motility in pre-and post-filtrated semen of each ejaculate was determined using research microscope supplied with a hot stage adjusted to 37°C. One drop from the diluted semen (1:20) was placed on a slide and was covered by a warmed cover slip and immediately examined under the high power magnification (x400). Number of motile spermatozoa was counted in field of a total of 100 sperm. Then percentage of sperm motility was calculated. Differences between pre-and post-filtered semen were calculated.

Statistical analysis

Regarding the evaluation of the raw semen, data were

statistically analyzed by the methods of least square analysis of variance using the general linear model procedures of SAS (1995). Data were statistically analyzed by method of paired analysis of variance using T-test according to Steel and Torri (1980).

The percentage values of sperm motility, live sperm and sperm abnormality were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

Results and Discussion

Evaluation of physical characteristics of raw semen

Regarding the differences between both species on basis of the overall means of the collection period (8 wk), only ejaculate semen volume was significantly ($P<0.001$) different, being higher in Holstein than buffalo bulls (5.64 and 3.15 ml, respectively, Table 1).

In spite of the insignificance of differences between the two species in other characteristics, there was tendency of higher percentages of motility, livability, abnormality and intact acrosome for spermatozoa in Holstein than buffalo bulls and tendency of higher sperm concentration in buffalo than Holstein bulls (Table 1).

Ejaculate volume was consistently higher in Holstein than buffalo bulls at all collection weeks (Fig. 1). These differences were insignificant at individual weeks, although the overall difference between the two species was significant ($P<0.001$, Table 1). Such insignificant differences at individual weeks may be attributed to individual variations in semen volume especially for Holstein bulls (Table 1). The highest volume of semen was recorded at the 2nd week in Holstein and at the 1st week in buffalo bulls (Fig. 1).

Using 3990 and 532 ejaculates from Friesian and buffalo bulls, respectively, El-Keraby *et al.* (1995) found

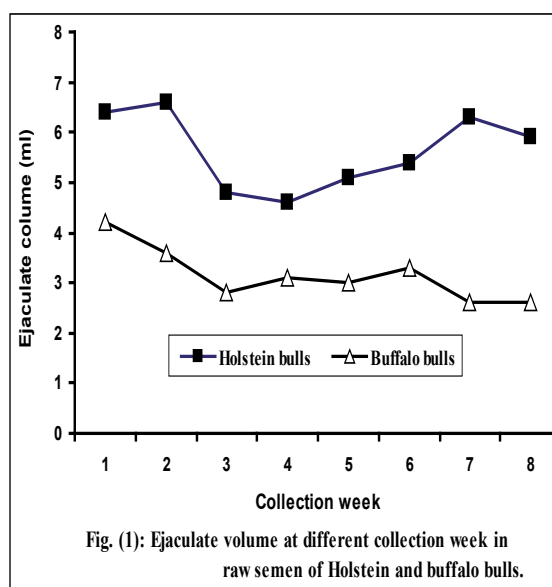


Fig. (1): Ejaculate volume at different collection week in raw semen of Holstein and buffalo bulls.

similar trends to that obtained in this study. They found that ejaculate volume increased significantly ($P<0.01$) in Friesian (4.0 ml) than buffalo (2.3 ml) bulls. In that study, percentage of motility of spermatozoa was significantly ($P<0.01$) higher in Friesian than buffalo bulls (71.5 vs. 63.0%).

The present study indicated that ejaculate volume in Holstein bulls was nearly double of that in buffalo bulls. This could be due to the variations in the functional capacity of the accessory reproductive glands, seminal vesicles, prostate gland and Cowper's gland between buffalo and Holstein bulls (El-Harairy *et al.*, 2005). The variation in ejaculate volume as affected by species reflected significant ($P<0.001$) changes in all terms of sperm output. So, the higher tendency of total sperm outputs in Holstein could be attributed to increased ejaculated volume during the experimental collection period.

Table 1. Physical characteristics of raw semen collected from Holstein and buffalo bulls.

Physical characteristics	Holstein semen	Buffalo Semen
Ejaculate volume (ml)	5.64±0.40 ^A	3.15±0.20 ^B
Sperm concentration ($\times 10^9$ /ml)	1.77±14.3	1.98±11.4
Sperm motility (%)	71.4±1.2	68.1±1.60
Live sperm (%)	73.0±1.3	72.8±1.40
Abnormal sperm (%)	4.3±0.50	3.9±0.30
Intact acrosome (%)	85.5±1.3	84.9±1.00

A and B: Means within the same row with the same superscripts are significantly different at $P<0.001$

Effect of filtration by sephadex column

Effect of filtration through sephadex column filter on different characteristics of Holstein and buffalo semen is shown in table (2). However, the comparison between the two species concerning the recovery rate of different characteristics as a result of filtration through sephadex column filter is illustrated in table (3).

In filtrate of both species, similar trend of change was observed in different semen characteristics resulting from filtration. Motility, livability and abnormality of spermatozoa were significantly ($P<0.001$) improved, whereas sperm cell concentration was significantly ($P<0.001$) reduced (Table 2).

The differences in all these traits between pre- and post-filtration when expressed as absolute values were similar in the two species (Table 3). However, when these differences were expressed as a post-filtration recovery rate, i.e. proportional to the pre-filtration values, Holstein

semen showed significantly ($P<0.05$) higher improvement of live sperm percentage and higher reduction in sperm cell concentration than those observed in buffalo semen (Table 3). Post-filtration recovery rate of motility and abnormality of spermatozoa did not differ significantly between the two species, although there were tendency of slightly more improvement in sperm motility and lower percentage reduction in sperm abnormality in buffalo than Holstein filtration.

Filtrates of both species in the present study contained approximately 80% sperm motility, 85% live sperm and 2% sperm abnormality, which are considered excellent values for semen that could be used for valuable insemination to ensure higher fertilization rate, as indeed the case *in vitro* fertilization trials. The present post-filtration motility values in the two species are in agreement with those obtained by Graham and Graham (1990) in bovine semen using sephadex G-15. Similar trend has been reported when bovine serum albumin gradients were used to filtrate semen of bull (White

Table 2. Effect of sephadex filtration on different characteristics of Holstein and buffalo semen.

Semen characteristics	Filtration		±MSE	Change	T-Value
	Pre-	Post-			
Holstein semen:					
Sperm motility (%)	64.5	80.9	1.2	+16.4	10.9***
Live sperm (%)	75.9	87.7	0.96	+11.8	18.1***
Abnormal sperm (%)	4.5	1.9	0.21	-2.6	11.1***
Sperm concentration (x10 ⁶ /ml)	6.3	3.0	0.11	-3.3	24.3***
Buffalo semen:					
Sperm motility (%)	63.5	80.4	1.4	+16.9	13.7***
Live sperm (%)	76.6	86.7	0.95	+10.1	16.8***
Abnormal sperm (%)	4.4	2.0	0.27	-2.4	10.1***
Sperm concentration (x10 ⁶ /ml)	7.1	3.6	0.24	-3.5	25.6***

*** T-value is significant at $P<0.001$

Table 3. Post-filtration recovery rate of different characteristics as affected by sephadex filtration of Holstein and buffalo semen.

Semen characteristics	Post-filtration recovery rate		±MSE	Significance
	Holstein semen	Buffalo semen		
Sperm motility (%)	25.4	26.6	1.1	NS
Live sperm (%)	15.5	13.2	0.80	*
Abnormal sperm (%)	- 57.8	- 54.5	1.26	NS
Sperm concentration (x 10 ⁶ /ml)	-52.4	-49.3	0.41	*

NS: Not significant

* Significant at $P<0.05$

Post-filtration recovery rate = Post filtration – Pre-filtration/ Pre-filtration x100

et al., 1984), boar (Dixon *et al.*, 1980), Stallion (Goodeaux and Kreider, 1978) and rabbit (Zavos, 1985).

In spite the beneficial impact of filtration of semen through sephadex column filter, the present post-filtration recovery rate of sperm motility was lower than that obtained for bovine semen using sephadex G-15 (Graham and Graham, 1990) or sephadex ion-exchange filter (Anzar and Graham, 1993).

This difference may be attributed to variations in quality of pre-filtration semen or the experimental procedures used in filtration. Maki-Laurila and Graham (1968) reported higher post-filtration recovery rate of sperm motility for bovine semen having poor sperm motility than that having high motility.

Also, Graham and Graham (1990) found that filtration of bovine semen with high percentage of abnormal spermatozoa resulted in higher recovery rate of motile and normal spermatozoa.

The mechanism of separation of immotile, dead and abnormal spermatozoa was suggested by Graham *et al.* (1976), who mentioned that filtration of spermatozoa on sephadex column appears to be physico-chemical reaction with sephadex particles providing a barrier, allowing these types of spermatozoa to agglomerate.

The present higher recovery rate of sperm motility than sperm livability and abnormality (Table 3) was related mainly to separation of immotile spermatozoa in addition to dead and abnormal spermatozoa from motile, live and normal spermatozoa, which pass through sephadex column. It was speculated that after the death of spermatozoa, positively charged components appear on the sperm membrane, which interact with negatively charged sephadex particles and are trapped. However, the live spermatozoa usually have a net negative surface charge bound to their plasma membrane and pass (Hammerstedt *et al.*, 1979 and Holt, 1980). On the other hand, damage to membranes of immotile and abnormal spermatozoa may lead to the exposure of different macromolecules, which might bind to the sephadex particles (Lodhi and Crabo, 1984).

Graham and Graham (1990) found that filtration of bull semen with high percentage of dead spermatozoa leads to significant increase in sperm motility and reduction in sperm concentration. The present results indicate that using sephadex column filter was more useful in improving sperm livability in Holstein than buffalo semen. Graham and Graham (1990) reported that sephadex filtration can

improve semen quality of low-0-fertile, but the fertility is still to be lower than that of the fertile bulls.

Conclusion

Sephadex filtration can improve semen quality of Holstein and buffalo bulls, but it was more useful in improving sperm livability in Holstein than buffalo semen.

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جودة السائل المنوي لطلائق الهولستين والجاموس المصري بعد ترشيحه باستخدام عمود السفدكس

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المخلص

أجريت هذه الدراسة في المركز الدولي للتدريب على رعاية الحيوان بسخا التابع لمعهد بحوث الإنتاج الحيواني - مركز البحوث الزراعية - وزارة الزراعة. ولقد هدفت الدراسة مقارنة نتائج ترشيح السائل المنوي المخفف لكل من طلائق الهولستين والجاموس المصري خلال عمود السفدكس. ولقد أجريت الدراسة على خمس طلائق هولستين (٧-١٠ سنوات) وخمس فحول جاموس (٩-١١ سنة) وجميع الحيوانات كانت سليمة صحيا وخالية من أي أمراض وعالية الخصوبة، حيث تم جمع السائل المنوي من كل منها لمدة ثمانية أسابيع بمعدل قذفين أسبوعيا ولقد أجريت جميع الاختبارات على درجة حرارة ٣٧°م. أدت عملية الترشيح إلي زيادة معنوية في النسبة المئوية للحيوانات المنوية المتحركة والحية بمعدل ١٦,٤ و ١١,٨٪ في الهولشتين وبمعدل ١٦,٩ و ١٠,١٪ في الجاموس، على الترتيب وانخفاض معنوي في النسبة المئوية للحيوانات المنوية الشاذة وتركيزها بمعدل ٢,٦ و ٣,٣٪ في الهولشتين وبمعدل ٢,٤ و ٣,٥٪ في الجاموس، على الترتيب. زاد معدل التحسن معنويا بعد الترشيح في نسبة الحيوانات المنوية الحية بدرجة أكبر في الهولستين عن الجاموس (١٥,٥٪ مقابل ١٣,٢٪) بينما لم تكن هناك اختلافات في معدل تحسن كل من النسبة المئوية للحيوانات المنوية المتحركة (٢٥,٤٪ مقابل ٢٦,٦٪) والشاذة (٥٧,٨٪ مقابل ٥٤,٥٪) في كلا النوعين، على الترتيب. ولقد أكدت الدراسة أن ترشيح السائل المنوي المخفف بواسطة عمود السفدكس يحسن جودة السائل المنوي عن طريق زيادة نسبة الحيوانات المنوية المتحركة والحية وتقليل نسبة الحيوانات المنوية الشاذة والميتة.