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Post-thaw Quality of Modified Dextran Swim-up Separated Bull's Semen Fractions

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ABSTRACT

Development of alternative, less harmful and feasible sperm sexing procedures through swim-up technique are cost-effective and highly desirable for domestic animals, especially bulls. Hence, the study was planned to assess fresh and post-thaw sperm quality after dextran swim-up separation. Semen was collected from four bulls to evaluate sperm quality, and separated fractions were divided into two groups (10 and 15 min.) according to the time allowed for swim-up separation using dextran transition layer and later cryopreserved using Tris-based extender. It was observed that concentration of spermatozoa and progressive motility% significantly ($P<0.05$) and motility% insignificantly ($P>0.05$) decreased in all fractions of two groups in comparison to fresh whole semen. We found significantly ($P<0.05$) the highest concentration of spermatozoa in Fraction 1 in both time groups. No significant ($P>0.05$) difference was observed in different parameters except motility% of Fraction 4 of two groups. Notably, there was no significant ($P>0.05$) difference found in kinematic parameters of fractioned semen collected at 10- and 15-minutes interval. After freezing and thawing, motility% and progressive motility% significantly ($P<0.05$) decreased in all fractions irrespective of groups. The highest post-thaw motility (45.57±7.54) and progressive motility% (5.42±1.50) was observed in Fraction 2 and Fraction 4 of 15 min group, respectively. This preliminary study could help animal andrologist to design sex pre-selective semen preparation protocol in a cost-effective way.

Introduction

Sperm selection is essential to obtain spermatozoa of good quality and high density from fresh semen for assisted reproductive technology (Hammerstedt *et al.*, 1990). Spermatozoa separation techniques are capable of barrier and removing damaged spermatozoa during the freeze-thaw process (Jaroudi *et al.*, 1993). The swim-up technique is a washing method to separate from seminal plasma and obtain a highly enriched population of motile and viable spermatozoa. The ability of spermatozoa to swim up from the ejaculate into the overlying medium has been used extensively as a method for separating as the population of highly motile spermatozoa (Berger *et al.*, 1985). It is one of the sperm preparation methods being used to separate the potentially fertile spermatozoa from immotile spermatozoa, debris, cryoprotectants, seminal plasma by active migration of the spermatozoa. In the swim-up techniques, motile spermatozoa migrate from the under layered medium (sperm specimen) and migrate across the sperm isolation medium interface to isolate themselves from the original sperm population (Fulgham and Alexander, 1990; Zavos, 1992).

Cryopreservation of semen has become a valuable technique for facilitating extensive utilization of frozen semen from genetically superior male animals (Evans and Maxwell, 1987). The cryoprotectants are added to extenders to protect the sperm from damage during the freezing process (Singh *et al.*, 1995). The success of cell cryopreservation protocols depends on two antagonistic factors: a low cooling rate that avoids intracellular ice formation and a short-time exposure to high osmolarity media that may cause irreversible membrane damage (Benson *et al.*, 2012). A critical factor in sperm cryobiology is that they are small cells with a large surface area (John Morris *et al.*, 2012). These characteristics affect the viscosity and glass transition temperature of the intracellular cytosol in sperm cells, which makes them less susceptible to potential damage (Isachenko *et al.*, 2002). Therefore, the research was performed to evaluate the quality of fresh and frozen

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swim-up separated fractions concerning collection time by studying kinetic parameters of semen using CASA.

Materials and Methods

Research duration and place

The experiment was performed from January 2019 to May 2019 at Research Animal Farm, Department of Surgery and Obstetrics, Bangladesh Agricultural University (BAU).

Experimental animals

Semen was collected from pure (100%) Holstein-Friesian (Harison), pure (100%) Sahiwal (Bahadur), 87.5% Holstein-Friesian crossbred (George) and 87.5% Sahiwal (Sultan) crossbred bulls.

Experimental procedure

Modified human tubular fluid and 1% dextran solution were used as separation media. After semen collection, the routine evaluation was performed along with the kinetic velocities using computer-assisted semen analyzer. Four fractions were collected following 10 minutes and 15 minutes interval. Samples were grouped according to the time of collection. Each fraction was evaluated for their kinematic parameters and plasma membrane functional integrity. Tris-based Media was prepared using Tris (2.42%w/v), citric acid (1.36%w/v), fructose (1%w/v), 20% (v/v) egg yolk, glycerol 7% (v/v), penicillin (100,000 IU), streptomycin (100 mg) and distilled water. The media was added in an equal volume (1:1) with the separated fractions for cryopreservation in one step method. Preservation was performed at -1960C for further evaluation in 0.25 ml straws. After thawing, semen of each fractions were evaluated for the kinematic parameters along with the motility% and progressive motility% using CASA.

Data analysis

Data was recorded in excel worksheet obtained from CASA software. Then the statistical analysis was performed by paired t-test to find out the comparison in each parameter between the fresh and frozen condition of semen within a specific time interval with SPSS 20.0.

Results

The general characteristics regarding concentration (×106 per ml), total motility %, progressive motility % and functional integrity of plasma membrane % of fresh semen samples used for the swim-up separation presented in Table 1. Kinetic velocities of fresh swim-up separated fractioned semen such as ALH (µm), BCF (Hz), LIN (%), VAP (µm/s), VCL (µm/s), VSL (µm/s) presented in Table 2. Separated fractions were divided into two groups (10 minutes and 15 minutes) according to the time allowed for swim-up separation using dextran transition layer. Four fractions were collected and evaluated in each group. The comparison among semen quality parameters of fresh whole and fractioned samples is also shown in Table 1.

It was observed that concentration of spermatozoa and progressive motility% significantly ($P<0.05$) decreased and motility% insignificantly ($P>0.05$) decreased in all fractions of two groups in comparison to fresh whole semen. But plasma membrane integrity% insignificantly ($P>0.05$) increased in Fraction 3 and Fraction 4 of 10 minutes group and Fraction 1 of 15 minutes group when compared with that of fresh whole semen. Among kinetic parameters, ALH, BCF, LIN and STR increased, and VCL and VSL decreased in all fractions of two groups in comparison to that of fresh whole semen.

Considering the fractions within groups, we found significantly ($P<0.05$) the highest concentration of spermatozoa in Fraction 1 in both time groups. Considering the motility% and progressive motility%, the parameters were the highest in Fraction 4 of 10 minutes group and Fraction 2 in 15 minutes group. On the other hand, we observed the highest value of plasma membrane functional integrity% in Fraction 4 in 10 minutes group and Fraction 1 of 15 minutes group. However, no significant ($P>0.05$) difference was observed in different parameters except motility% of Fraction 4 of two groups. Regarding the kinetic velocities of fractions, we found the highest ALH in Fraction 2 and Fraction 1 of 10 and 15 minutes groups respectively; LIN and STR in Fraction 3 of two groups; BCF, VAP, VCL and VSL in Fraction 3 and Fraction 4 of 10 minutes and 15 minutes groups respectively. Importantly, there was no significant ($P>0.05$) difference found in kinematic parameters of fractioned semen collected at 10 and 15 minutes interval (Table 2).

Comparison between fresh and frozen fractions

In this study, all fractioned samples were frozen after one step dilution with Tris-based extenders at 1:1 dilution rate. Table 3, Table 4, Table 5, Table 6 shows the variation between fresh and frozen-thawed semen parameters of Fraction 1, 2, 3, and 4, respectively. Motility% and progressive motility% significantly ($P<0.05$) decreased in all fractions irrespective of groups. ALH and BCF insignificantly ($P>0.05$) increased in all Fractions of 10 minutes group. LIN and STR significantly ($P<0.05$) decreased in Fraction 3 (Table 5) and VCL, VAP and VSL significantly ($P<0.05$) decreased in Fraction 2 (Table 4) and Fraction 4 (Table 6) of 15 minutes group. The highest Post-thaw motility (45.57±7.54) and progressive motility% (5.42±1.50) were observed in Fraction 2 and Fraction 4 of 15 minutes group, respectively (Table 4 and Table 6).

Table 1. General parameters (Mean± SEM) of fresh whole and Fractions obtained at different times

Parameter	Fresh whole	Interval	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Concentration (million/ml)	1617.50±323.16	10	328.84±54.99	104.80±44.94	114.15±68.26	119.17±92.37
		15	251.62±91.26[a]	126.66±48.20[b]	91.99±40[b]	59.20±24.20[b]
Motility %	83.42±5.62	10	67.25±7.12	61.6±9.73	63±4.41	72.42±3.53*
		15	61.85±3.03	66.55±0.78	54.42±6.98	47.42±9.14
Progressive motility %	54.55±6.03	10	33.5±9.21	31.15±5.02	36.32±12.64	41.07±11.21
		15	28.75±3.96	34.25±6.64	32.65±10.48	33±10.11
PM Functional integrity %	48.25±4.33	10	37.5±3.4	40.25±2.32	58.5±3.48	63.5±2.38
		15	53±1.29	49.75±1.79	32.25±3.12	30.25±4.97

Within a single row, values with different superscripts (a, b) differ significantly among fractions and within a single column, (*) differ significantly within minutes (P<0.05).

Table 2. Kinematic parameters of fresh whole and Fractions obtained at different times

Parameters	Fresh whole	Interval	Fraction 1	Fraction 2	Fraction 3	Fraction 4
ALH	8.09±2.07	10	6.98±0.50	7.26±1.16	6.43±1.36	6.48±0.52
		15	7.51±0.36	6.34±0.62	6.19±0.66	6.96±0.98
BCF	28.98±7.85	10	36.07±2.94	33.45±3.06	36.58±3.14	33.02±2.51
		15	34.62±2.45	33.91±2.53	35.52±2.19	37.46±2.03
LIN	40.18±9.84	10	43.33±3.74	47.83±3.55	49.09±9.21	45.98±5.89
		15	42.21±2.95	47.11±4.18	48.28±6.06	47.78±4.86
STR	63.11±17.44	10	77.1±3.89	80.10±3.89	81.60±6.43	81.57±5.25
		15	76.27±2.48	80.84±2.10	83.28±4.23	82.19±5.14
VAP	114.09±27.55	10	69.50±6.08	80.27±7.68	82.83±15.38	74.25±11.39
		15	75.66±7.38	78.48±9.46	80.13±14.31	91.48±15.18
VCL	197.26±50.16	10	127.05±8.78	137.22±12.44	145.94±27.84	132.65±14.22
		15	139.88±9.26	134.92±8.87	138.43±20.38	161.89±31.02
VSL	94.55±20.45	10	54.80±6.43	65.45±6.51	69.29±15.46	63.15±11.71
		15	60.02±6.69	65.90±8.79	69.37±14.42	77.85±13.51

Within a single row, values with different superscripts (a, b) differ significantly among fractions and within a single column, (*) differ significantly within minutes (P<0.05).

Table 3. Kinetic and general parameters of Fraction 1

Parameters	Fraction -1			
	10 minutes		15 minutes	
	Fresh	Frozen	Fresh	Frozen
Concentration (million/ml)	328.84±54.99	237.94±135.92	251.62±91.26	113.83±46.39
Motility %	67.25±7.12	10.15±1.91**	61.85±3.03	17.95±5.92**
Progressive motility %	33.5±9.21	2.92±0.82*	28.75±3.96	4.45±1.30**
ALH	6.98±0.50	10.45±1.23	7.51±0.36	8.88±1.61
BCF	36.07±2.94	42.10±4.47*	34.62±2.45	36.81±2.91
LIN	43.33±3.74	31.24±3.51	42.21±2.95	36.01±2.99
STR	77.1±3.89	59.69±4.32	76.27±2.48	65.60±5.54
VAP	69.50±6.08	63.75±0.87	75.66±7.38	67.95±9.55
VCL	127.05±8.78	149.02±17.00	139.88±9.26	134.93±21.13
VSL	54.80±6.43	38.44±2.86	60.02±6.69	42.97±2.99

Within a single row, (*) indicates a significant difference at P<0.05 and (**) indicates a highly significant difference at P<0.01 within minutes.

Table 4. Kinetic and general parameters of Fraction 2

Parameters	Fraction -2			
	10 minutes		15 minutes	
	Fresh	Frozen	Fresh	Frozen
Concentration (million/ml)	104.80±44.94	60.95±3.34	126.66±48.20	59.49±20.14
Motility %	61.6±9.73	11.6±1.69*	66.55±0.78	45.57±7.54
Progressive motility %	31.15±5.02	3.2±0.71*	34.25±6.64	4.82±1.07*
ALH	7.26±1.16	10.40±1.29	6.34±0.62	5.39±0.27
BCF	33.45±3.06	42.52±4.99	33.91±2.53	33.21±1.92
LIN	47.83±3.55	31.19±2.42	47.11±4.18	37.59±5.27
STR	80.10±3.89	59.04±4.03	80.84±2.10	74.71±3.26
VAP	80.27±7.68	67.25±5.15	78.48±9.46	39.09±1.75*
VCL	137.22±12.44	145.79±17.45	134.92±8.87	81.09±4.09**
VSL	65.45±6.51	40.91±1.07*	65.90±8.79	27.18±2.78*

Within a single row, (*) indicates a significant difference at P<0.05 and (**) indicates a highly significant difference at P<0.01 within minutes.

Table 5. Kinetic and general parameters of Fraction 3

Parameters	Fraction -3			
	10 minutes		15 minutes	
	Fresh	Frozen	Fresh	Frozen
Concentration (million/ml)	104.80±44.94	68.45±25.17	126.66±48.20	57.51±24.71
Motility %	61.6±9.73	23.75±4.57**	66.55±0.78	14.2±6.04*
Progressive motility %	31.15±5.02	6.25±2.25*	34.25±6.64	2.60±0.76*
ALH	6.43±1.36	9.17±1.58	6.19±0.66	8.77±1.27
BCF	36.58±3.14	38.66±1.19	35.52±2.19	38.54±3.32
LIN	49.09±9.21	36.16±4.60	48.28±6.06	31.56±3.57*
STR	81.60±6.43	68.64±2.72	83.28±4.23	63.14±6.73*
VAP	82.83±15.38	63.72±9.95	80.13±14.31	53.72±7.40
VCL	145.94±27.84	132.02±24.98	138.43±20.38	117.50±16.31
VSL	69.29±15.46	43.70±6.86	69.37±14.42	34.06±5.55

Within a single row, (*) indicates a significant difference at P<0.05 and (**) indicates a highly significant difference at P <0.01 within minutes.

Table 6. Kinetic and general parameters of Fraction 4

Parameters	Fraction -4			
	10 minutes		15 minutes	
	Fresh	Frozen	Fresh	Frozen
Concentration (million/ml)	119.17±92.37	40.54±6.53	59.20±24.20	44.47±12.36
Motility %	72.42±3.53	17.67±3.43**	47.42±9.14	42.85±6.61
Progressive motility %	41.07±11.21	3.70±1.10*	33±10.11	5.42±1.50*
ALH	6.48±0.52	7.97±1.07	6.96±0.98	5.63±0.54
BCF	33.02±2.51	38.64±1.47	37.46±2.03	33.57±2.44
LIN	45.98±5.89	30.61±2.03*	47.78±4.86	37.33±4.65
STR	81.57±5.25	64.36±4.74	82.19±5.14	73.79±4.74
VAP	74.25±11.39	52.00±6.34	91.48±15.18	40.07±2.10*
VCL	132.65±14.22	114.39±12.46	161.89±31.02	81.62±4.55
VSL	63.15±11.71	32.74±3.61	77.85±13.51	27.77±4.08*

Within a single row, (*) indicates a significant difference at P<0.05 and (**) indicates a highly significant difference at P<0.01 within minutes.

Discussion

Swim-up separation techniques involve sperm separation depending on sperm motility characteristics. We separated four fractions in a hypothesis that population of X and Y spermatozoa might be variable present in different fractions according to their motility characteristics. In this study, no significant variation (P>0.05) within bull and effects of fraction were found for all quality parameters of sperm studied.

In this study, the efficacy of dextran-swim up sperm separation technique and research were conducted to examine the time effect on the quality of fresh and frozen swim-up whole separated fractions. Verstegen *et al*. (2002) have stated that motility is used as a common criterion in routine semen analysis, and it is considered as an expression of viability and structural integrity of spermatozoa. The values of motility% observed in fresh and fractioned semen are inconsistent with the reports of Mehmood *et al*. (2009) and Matas *et al*. (2011); who have stated that the motility of spermatozoa before sperm processing ranges from 38.5-70.0% and after sperm processing in swim-up method motility increased to 69.1-88.0%.

Among kinetic parameters, ALH, BCF, LIN and STR increased, and VCL and VSL decreased in all fractions of two groups in comparison to that of fresh whole semen in this study. In contrast, there are several reports on the Swim-up cleansing method which stated improved the VCL, VSL (Esteves *et al*., 2000; Person *et al*., 2007; Matas *et al*., 2011; Oliveira *et al*., 2011), VAP and VCL (Hallap *et al.*, 2004; Person *et al.*, 2007), LIN (%) (Esteves *et al.*, 2000), and decreased STR (%) and BCF (Person *et al.*, 2007; Gillan *et al.*, 2008; Oliveira *et al.*, 2011). However, the variations in motility% and kinetic values might have resulted from the use of different separation technique used in this study.

Conclusion

In this study, effects for all sperm quality parameters were studied within bulls and fractions. The concentration of spermatozoa and progressive motility% significantly (P<0.05) and motility% insignificantly (P>0.05) decreased in all fractions of two groups in comparison to fresh whole semen. After freezing and thawing, motility% and progressive motility% significantly (P<0.05) decreased in all fractions irrespective of groups. The dextran swim-up procedure is inexpensive and simple to perform. To maximize reproductive efficiency and the fertilizing ability of semen samples, a reduction in the number of spermatozoa used per insemination is an important objective in animal production. Therefore, we should design future study to freeze swim-up separated fractions in an acceptable and feasible way to produce

desired number of male and female offspring. We strongly believe that more studies should confirm the data before recommendations for widespread use of the modified procedure used in this study.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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