Relationship between Semen Quality Parameters and Field Fertility of Bulls

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ABSTRACT

A study was conducted on four crossbred bulls, used as artificial insemination (AI) sires, to correlate their semen quality with their non return rate (NRR). Semen was collected once a week via an artificial vagina, diluted in egg yolk-citrate and maintained at +7℃ for three days. It was evaluated for sperm motility, viability, morphology immediately after collection and was examined daily for sperm motility, viability and morphology of acrosome, mid piece and tail for a total of three days. A total of 2016 cows were inseminated by two AI technicians. The proportions of sperm with normal heads were 83.4% (63.7～91.7%), the proportion of spermatozoa exhibiting normal morphology (acrosome, mid piece and tail), motility and viability were 89.2% (82.3～92.0%), 71.3% (61.7～75.0%) and 76.7% (65.7～85.0%), respectively in fresh ejaculates. Sperm motility and sperm viability was significantly (p<0.05) lower in Holstein-Friesian × Local bull than in other bulls during all three days of storage. The overall NRR for four bulls was 82.7% (72.9-87.5%). Bulls with higher sperm motility, viability and normal morphology of spermatozoa of individual bull had significantly (each p<0.05) higher NRR. The highest (p<0.01) NRR (87.5%) was observed in a Red Chittagong bull whose semen qualities were significantly (p<0.05) higher than Holstein-Friesian × Local bull (NNR 72.9%). The results of the present study concluded that NRR at 56 days post AI is related to parameters of semen quality. Therefore, semen evaluation may allow the discarding of bulls with poor fertility in an AI program.

(Key words : non return rate, sperm motility, viability, sperm morphology)

INTRODUCTION

Pregnancy in cows from AI services is the key factor for optimal economic success in dairy farms. AI is the oldest and currently most common assisted reproductive technology and an important tool in animal production (Vishwanath, 2003). Failure of cows to become pregnant and the need for repeated AI are common causes of frustration and economic loss of the dairy farmers (Stevenson *et al.*, 1990). Since the aim of an insemination is to produce pregnancy, the fertilizing capacity of spermatozoa preserved either as chilled or as frozen should never be compromised in an AI program. To make an AI program into an economic success, sires with inadequate fertility (whether in terms in individual poor-quality ejaculates or of a bull whose conception rates are unacceptable) must be identified and removed (Shaha *et al.*, 2008). A national program for crossing local cattle with exotic breeds by AI has been practiced in Bangladesh since 1950 (Ahmed and Islam, 1987). Reproductive efficiency of AI bulls is usually measured by the NRR and it is measured on the cow. It is affected by the fertility qualities of the cow herself and of the bull she is inseminated by, and also by the season and skillness of the technicians who carried out the insemination (Reurink *et al.*, 1990). No other system except NRR to monitor male fertility has been introduced on a large scale (Reurink *et al.*, 1990).

Semen can be used in AI programs after cryopreservation, or after dilution in either ambient-temperature (e.g. Caprogen) or chilled diluents (Holt, 2000). Semen survives dilution and/or chilling for a few days, but the motility and morphology of spermatozoa progressively worsen as the period of storage increases (Alam *et al.*, 2005). Moreover, motility can also be affected by the age of the sire and environmental influences (Hallap *et al.*, 2006). However, no information exists on the performance of chilled semen in the national crossbreeding program of cattle breeding in Bangladesh, nor has the relationship between semen quality and NRR been evaluated for the bulls in this program. This study was, there fore, performed to identify low fertility bulls, on the basis of their semen qualities

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(sperm motility, head morphology, morphology of acrosome, mid piece and tail and viability of sperm) and NRR under field condition, so that such bulls can be discarded from a commercial bull AI center.

MATERIALS AND METHODS

The research work was conducted at the University AI Center and Reproduction Laboratory of the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

1. Bulls

Four healthy vaccinated, dewormed and sexually matured crossbred bulls were selected for this study. The age, body condition score and body weight of the bulls were 3～5.5 years, 3.5～4.0 (1 to 5 scales) and 345～561 kg, respectively.

2. Measurement of Scrotal Circumference

Scrotal circumference was measured in centimeter by using measuring tape (Lane Manufacturing Co., Denver, CO, USA) according to the method described by Foote (1969).

3. Semen Collection and Transportation

Semen was collected by artificial vagina (AV) once per week, following the method adopted by Islam *et al.* (1999). The semen was maintained at +37℃ after collection until evaluation and processing.

4. Semen Evaluation

The routine evaluation of fresh semen was done as described by Alam *et al.* (2005) and Shaha *et al.* (2008). Volume of fresh semen was recorded from the graduated mark of the semen collecting tube. Mass activity was evaluated by placing a drop (25 μl) of semen onto a pre-warmed (+37℃) glass slide without cover slip and examined under light microscope at low magnification (100×). The concentration of spermatozoa (million/ml) was determined by using haemocytometer technique as described by Bane (1952).

Sperm motility was assessed by observing motion under 100× magnifications using 10× optical systems. Sperm head morphology was evaluated in dried semen smears using differential interference contrast (DIC) optics (BX 51, OLYMPUS, Tokyo Japan; 1000× magnification) as previously described by Freneau *et al.* (2009). Morphology of sperm acrosome, mid-piece and tail was examined using samples diluted in buffered formal saline as method described by Barth and Oko (1989). Sperm viability (proportions of live and dead spermatozoa) was estimated by using Eosin-Nigrosin staining (Evans and Maxwell 1990).

5. Semen Processing and Preservation

Semen was processed in egg-yolk-citrate (EYC) extender (Jha, 2008). After collection, the semen was diluted with EYC to obtain 20×10^6 progressive motile spermatozoa per ml. Individual insemination doses were transferred into one ml glass vials, which were preserved at +7℃ for three days for artificial insemination.

6. Insemination

Inseminations of cows were done at 12～18 hours of onset of estrus by two inseminators of University AI center. Cows showing any abnormal genital discharge or having any abnormalities in genital organs on rectal palpation at the time of AI were not considered for this experiment.

7. Data Collection

Data for individual insemination records were collected from the registers books of University AI center. Data including the bull whose semen used, date of semen collection and insemination, identification of the cow which received insemination, and where applicable, the date on which cows were represented for insemination after return to estrus by 56 days post-insemination were recorded.

8. Calculation of Non Return Rate

Non return rate was calculated by the formula given below:

$$NNR = \frac{\text{Total no. of cows inseminated} - \text{No. of cows returned to estrus}}{\text{Total no. of cows inseminated}} \times 100\ \%$$

9. Statistical Analysis

Data were entered in a Microsoft excel work book and exported to SPSS (Version 10.0) for analysis of descriptive statistics. Linear regression was performed to figure out the relationship between qualities of fresh semen and NRR of bulls. one way ANOVA was used to see the effects of individual bulls on semen volume, sperm concentration, sperm motility, number of live sperm of fresh semen, and the changes of sperm

motility and viability from Day 1 to 3 (on each day) of preservation among the bulls.

RESULTS

The age, body condition score, body weight and scrotal circumference of the bulls were 3～5.5 years, 3.5～4.0 (1 to 5 scales), 345～561 kg and 26～32 cm, respectively. Details of the bulls used in this study are given in Table 1.

Semen quality data for the four bulls are presented in Table 2. The mean volume of fresh semen collected from four crossbred bulls varied from 3.9 ± 0.4 to 7.7 ± 0.3 ml. There were significant differences between bulls in ejaculate volume ($p<0.05$). The sperm concentrations ($\times 10^6$/ml) of fresh semen ranged between 1,116.7 ± 28.9 to 1,315.0 ± 13.2 among the bulls. The sperm concentrations are significantly differed among the bulls ($p<0.05$). The sperm motility (%) of fresh semen varied from 61.7 ± 2.9 to 75.0 ± 5.0. The differences of sperm motility among the bulls are statistically significant ($p<0.05$). The proportions of live spermatozoa in fresh ejaculates varied from 65.7 ± 4.0 to 85.0 ± 1.0% among the four bulls and this difference is statistically significant ($p<0.05$).

Examples of different sperm head abnormalities are shown in Fig 1. The percentages of spermatozoa with normal heads varied from 63.7 ± 3.2 to 91.7 ± 1.6 among the bulls. Significant ($p<0.05$) differences in sperm head morphology were present between bulls (Table 3). Examples of different abnormalities of sperm acrosome, mid-piece and tail, are shown in Fig. 2. The proportions of spermatozoa with normal acrosomes, mid-pieces and tails were also differed significantly ($p<0.05$) among the bulls, which varied from 82.3 ± 2.5 to 92.0 ± 1.5% (Table 3).

Sperm motility and sperm viability both decreased significantly ($p<0.05$) as period of storage increases (Table 4). The number of spermatozoa with abnormal acrosomes, mid-pieces and tails were significantly ($p<0.05$) increased with the advancement of time of preservation. Sperm motility and sperm viability was also significantly ($p<0.05$) lower in Holstein-Friesian × Local bull than in other bulls on all three days.

A total of 2,016 cows were inseminated, of which 349 cows were presented for second insemination. Non return rate for each bull is presented in Table 5. The overall NNR is 82.7% (72.9～87.5%) for four bulls. The highest NRR was found in Red Chittagong bull that was significantly ($p<0.01$) higher with

Table 1. Brief description of bulls used at AI center

Bull ID No.	Breeds	Age (Years)	Body condition score (1 to 5 scale)	Body weight (kg)	Scrotal circumference (cm)
131	Holstein-Friesian × Local	4.5	4.0	495	30
143	Sahiwal × Local	3.5	4.0	450	28
122	Sindhi × Local	5.5	4.0	561	32
092	Red Chittagong	3.0	3.5	345	26

Table 2. Evaluation of fresh semen (volume of semen, mass activity, motility and viability of sperm) of bulls. Number of ejaculates per bull=3

Bull ID	Volume (ml)	Mass activity (1 to 4 scales)	Sperm conc. (10^6/ml)	Motility (%)	Live spermatozoa (%)
131	3.9 ± 0.4[a]	3 (2 to 4)	1,219.0 ± 26.9[b]	61.7 ± 2.9[a]	65.7 ± 4.0[a]
143	5.6 ± 0.1[c]	3 (3 to 4)	1,116.7 ± 28.9[a]	75.0 ± 5.0[b]	73.7 ± 1.5[b]
122	7.7 ± 0.3[d]	3 (2 to 4)	1,315.0 ± 13.2[d]	75.0 ± 5.0[b]	82.3 ± 2.5[c]
092	4.6 ± 0.4[b]	3 (2 to 3)	1,256.7 ± 5.6[c]	73.3 ± 2.9[b]	85.0 ± 1.0[c]
Pooled	5.5 ± 1.7		1,226.9 ± 83.4	71.3 ± 6.4	76.7 ± 8.8

Data are expressed as Mean ± SD.
[a～d] Values within the same column with different superscripts differ significantly from each other ($p<0.05$).

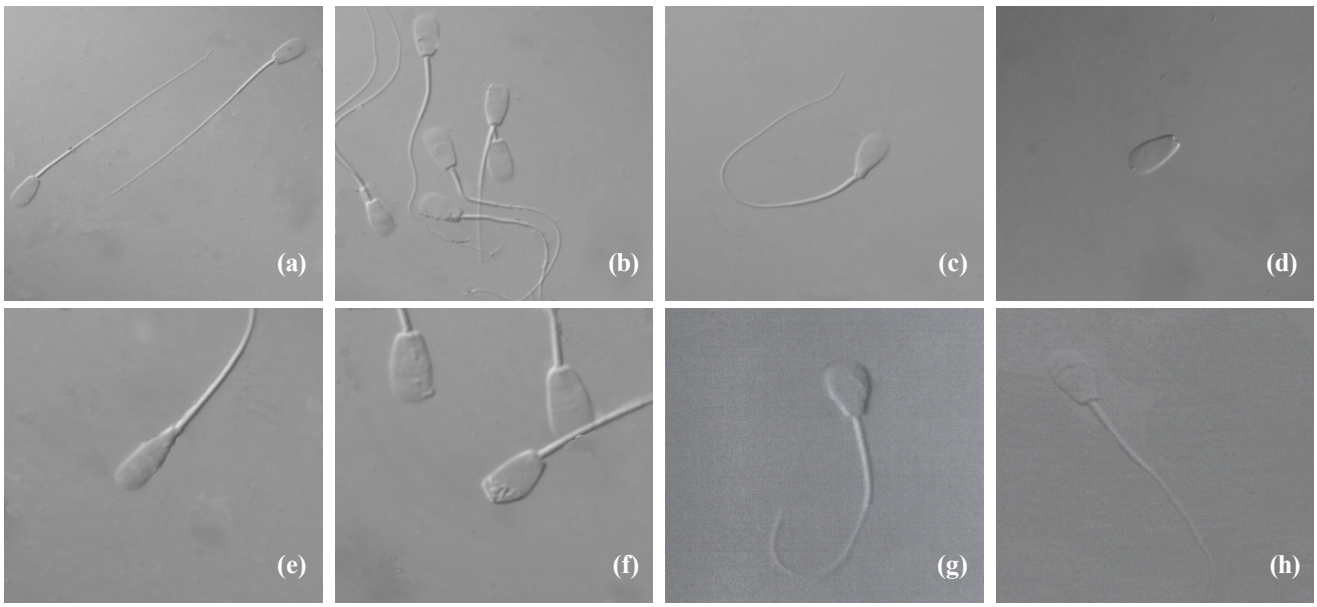

Fig. 1. Photography of abnormal morphology of bull sperm head observed at DIC optics (1,000× magnification). (a) normal sperm head, (b) small head, (c) pyriform head, (d) detached head, (e) narrow head, (f) abnormal shaped head, (g) pear shaped head, (h) damaged acrosomal membrane.

that of Holstein Friesian × Local which achieved 72.9% NNR.

Bulls with semen of higher sperm motility in fresh ejaculates had significantly ($R^2 = 0.63$, $p<0.05$) higher NNR (Fig. 3a). Bulls having large number of viable spermatozoa in fresh semen achieved significantly ($R^2 = 0.73$, $p<0.05$) higher NRR (Fig. 3b). The proportion of sperm with normal head morphology was significantly ($p<0.05$) correlated to NRR ($R^2 = 0.90$, Fig. 3c) in cows, whilst the number of sperm with normal acrosomes, mid-

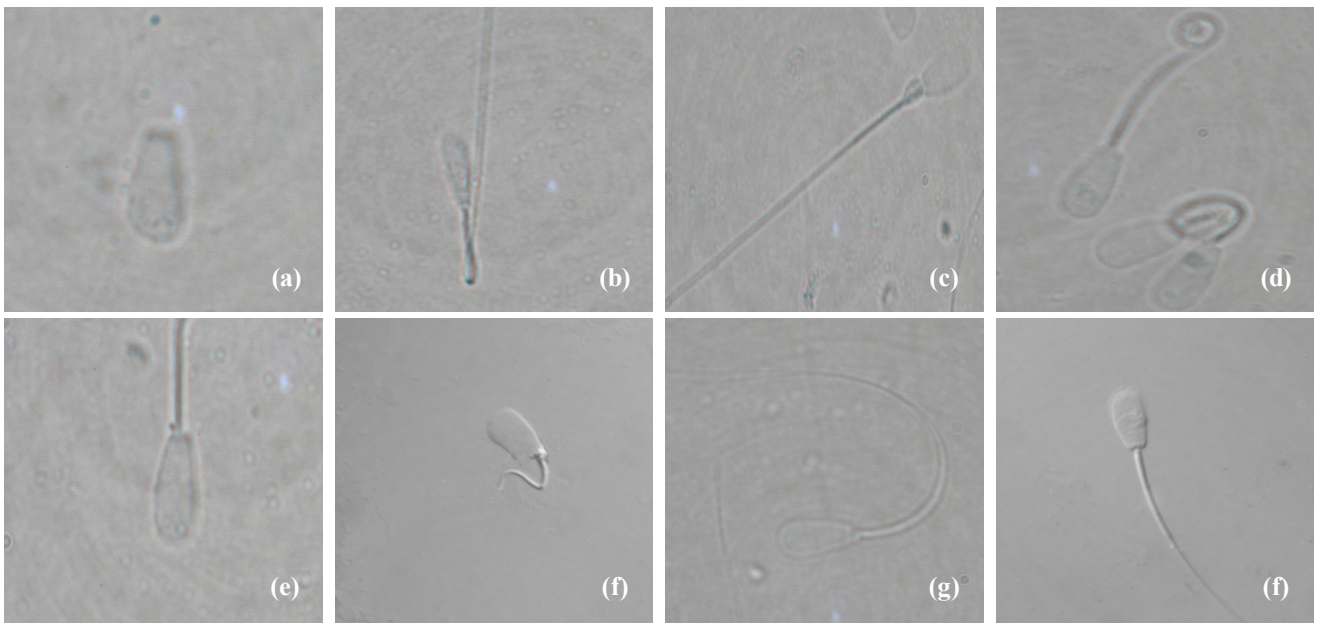

Fig. 2. Photography of abnormal acrosome, mid-piece and tail of bull sperm observed at 1,000× under DIC optics. (a) detached head, (b) broken of mid-piece, (c) proximal cytoplasmic droplet, (d) cork screw shape tail, (e) broken tail, (f) abaxial tail, (g) simple bent tail, (h) loose acrosome.

Table 3. Evaluation of fresh semen (sperm morphology) of bulls. Number of ejaculates for each bull = 3

Bull ID	Sperm with normal head (%)	Sperm with normal acrosome, mid piece and tail (%)
131	63.7 ± 3.2^{a}	82.3 ± 2.5^{a}
143	90.0 ± 2.0^{bc}	91.3 ± 1.5^{b}
122	88.0 ± 2.0^{bc}	91.0 ± 1.0^{b}
092	91.7 ± 1.6^{c}	92.0 ± 1.5^{b}
Pooled	83.4 ± 13.2	89.2 ± 4.6

Data are expressed as Mean ± SD.
[a~c] Values within the same column with different superscripts differ significantly from each other ($p<0.05$).

pieces and tails was significantly ($p<0.05$) correlated to NRR ($R^2 = 0.93$, Fig. 3d).

DISCUSSION

The results of the present study showed that non return rate of bulls was significantly related to their semen quality, such that the bulls which given semen of higher sperm motility and viability and lower proportion of morphologically normal spermatozoa achieved higher NRR.

The mean volume of fresh semen collected from four crossbred bulls varied from 3.9 ± 0.4 to 7.7 ± 0.3 ml, which is consistent with the other studies of bulls in Bangladesh (Bhuiyan and Shamsuddin, 1998; Jha, 2008). Similarly, Munsi *et al.* (2007) reported that the fresh semen volume of crossbred bulls ranged between 2.9 ± 0.2 to 4.4 ± 0.5 ml. The sperm concentrations ($\times 10^6$/ml) of fresh semen varied from $1,116.7 \pm 28.9$ to 1315.0 ± 13.2, which were consistent with the results of Munsi *et al.* (2007) and Jha (2008). The sperm motility (%) of fresh semen varied from 61.7 ± 2.9 to 75.0 ± 5.0, which again is in agreement with previous studies in Bangladesh (Sugulle *et al.*, 2006; Munsi *et al.*, 2007; Jha, 2008). Finally, the use of DIC microscopy (range: $63.7 \pm 3.2\%$ to $91.7 \pm 1.6\%$) to evaluate sperm head morphology gave results that were comparable with earlier studies (Sugulle *et al.*, 2006; Siddiqui, 2007; Freneau *et al.*,

Table 4. Changes of semen quality on Days 1 to 3 after preservation at +7℃

Bull ID	Day 1	Day 2	Day 3
	Sperm motility		
131	61.7 ± 2.9^{a}	51.7 ± 2.9^{a}	41.7 ± 2.9^{a}
143	75 ± 5^{b}	62.3 ± 2.5^{b}	47.7 ± 2.5^{b}
122	75 ± 5^{b}	61.7 ± 2.9^{b}	43.3 ± 2.9^{ab}
092	73.3 ± 2.9^{b}	56.7 ± 5.8^{ab}	43.3 ± 2.9^{ab}
	Sperm viability		
131	65.7 ± 4^{a}	55.7 ± 2.1^{a}	43 ± 2.7^{a}
143	73.67 ± 1.6^{b}	64 ± 1^{b}	46.3 ± 1.6^{ab}
122	82.3 ± 2.6^{c}	70 ± 2^{c}	47.7 ± 1^{b}
092	85 ± 1^{c}	66.3 ± 2.1^{b}	50 ± 2^{b}
	Sperm with normal acrosome, mid piece and tail		
131	82 ± 2.5^{a}	80 ± 2^{a}	77.7 ± 1.5^{a}
143	91 ± 1.5^{b}	89 ± 1.7^{b}	87 ± 1.7^{b}
122	91 ± 1^{b}	88 ± 1.7^{b}	85 ± 1^{b}
092	92 ± 1.5^{b}	88.7 ± 1.5^{b}	86 ± 1.7^{b}

[a~c] Values within the same column with different superscripts differ significantly from each other ($p<0.05$).

Table 5. Non return rates of bulls

Bull ID	Breeds	No. of cows inseminated	No. of cows represented for insemination	NRR (%)	OR (CI)	*p* value
131	Holstein Friesian × Local	532	144	72.9*	0.374 (0.183~0.7630)	0.007
143	Sahiwal × Local	500	70	86.0	0.793 (0.392~1.606)	0.520
122	Sindhi × Loca	544	80	85.2	0.747 (0.371~1.505)	0.415
092	Red Chittagong	440	55	87.5	1	–
Total		2,016	349	82.7		

OR = odds ratio, CI = confidence interval.
* Indicates significant ($p<0.01$).

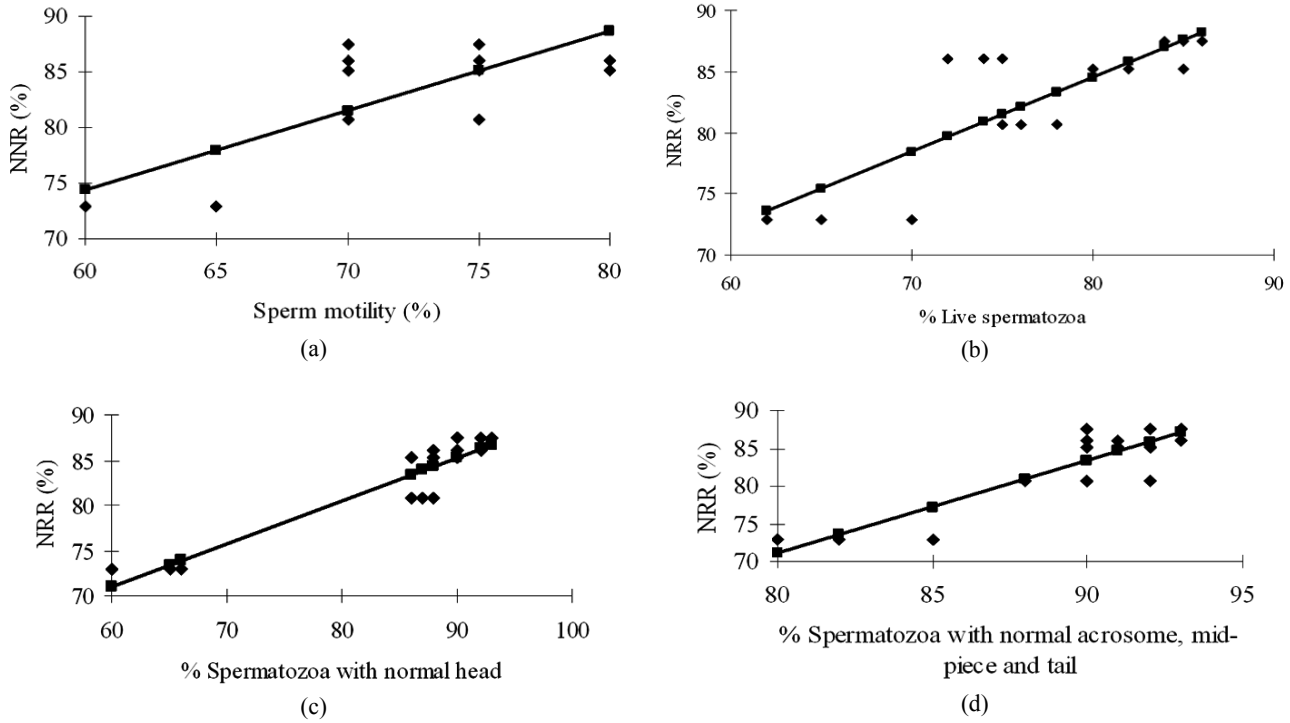

Fig. 3. Relationship between fresh semen quality and NNR of bulls. (a) sperm motility; R^2 = 0.63, p<0.05, (b) percentages of live spermatozoa; R^2 = 0.73, p<0.05, (c) percentages of sperm with normal head; R^2 = 0.90, p<0.05, (d) percentages of sperm with normal acrosome, mid-piece and tail; R^2 = 0.93, p<0.05.

2009). As previously reported, there is a significant level of variation between bulls and between ejaculates, which has been variously attributed to age, season, technique of semen collection, body weight and scrotal circumference of the bulls (Helbig *et al.*, 2006; Siddiqui *et al.,* 2007), whilst morphology can also vary with processing and storage of semen, diluents and duration of storage (Shamsuddin and Chanda, 1998). Semen is described normal when the frequency of abnormal sperm heads does not exceed 10% and other parameters (acrosome, mid-piece, and tails) exceed 5% each or a total of 10～15% (Rodriguez-Martinez, 2008).

There was significant difference in the proportion of sperm with abnormal head morphology among the bulls evaluated by DIC microscopy. The process of evaluating head morphology is easier using DIC than using William's staining technique. According to Freneau *et al.* (2009), DIC is a more effective technique for visualizing major defects, although bright field, which included stained smear preparations may be preferable for visualizing minor defects.

In the present study, sperm motility and viability decreased gradually over three days of preservation at 7℃. This trend has been widely reported in the international literature on chilled semen diluents, and is also in agreement with the results of Jha (2008) in Bangladesh. Provided at least 50% of sperm display progressive motility, the semen can be used for AI. Previous work has shown that semen can routinely be maintained at this level of viability in egg yolk-citrate diluent at 7℃ for two days (Alam *et al.*, 2005) in Bangladesh AI programmes.

Non return rate was significantly related to semen quality, such that the bulls with semen of higher motility and viability and lower proportion of morphologically abnormal sperm had higher NRR. Sarder (2006) similarly reported that the highest NRR (83.1%) was associated with high quality semen and the lowest (70.1%) with poor quality bull semen in the Rajshahi district. The evidence of both the present experiment and the literature is that the variation in NRR between bulls and/or ejaculates is due to differences in the proportion of morphologically normal motile spermatozoa in the insemination dose. Thus, there was a significant (p<0.01) relationship (R^2 = 0.63) between sperm motility of fresh semen and NRR; a finding that is in agreement with previous studies. For example, Amann and Hammerstedt (2002) reported that both the number of

motile sperm per dose as well as the number of viable sperm per dose correlated significantly ($p<0.01$) with NRR ($r = 0.49$, and $r = 0.83$, respectively). Likewise, Bhuiyan *et al.* (1999) reported that cows inseminated with good quality bull semen (i.e. > 50% motility, > 7.5×10^6 total number of motile spermatozoa and >70% normal sperm) in an AI programme in Bangladesh that used chilled semen, conceived at higher rate ($p< 0.001$) than did those inseminated with poor bull semen (55.2% vs 37.1%). In the present experiment, a significant ($p<0.05$) relationship between sperm viability and NRR was observed ($R^2 = 0.73$), which again is in accordance with previous studies, such as that of Decuadro-Hansen *et al.* (2002) who reported that sperm viability and sperm motility correlates with the fertilizing ability of a particular sample of semen. Similarly, in the present study, the proportion of sperm with normal head morphology was significantly ($p<0.05$) correlated to NRR ($R^2 = 0.90$) in cows, whilst the number of sperm with normal acrosomes, mid-pieces and tails were significantly ($p< 0.05$) correlated to NRR ($R^2 = 0.93$). Al-Makhzoomi *et al.* (2008) reported that sires' having ejaculates containing >10% of morphologically deviating sperm head shapes are associated with poor NRR. Card (2005) suggested differential spermiogram that included less than 30% morphologically normal spermatozoa, more than 10% immature germ cells, more than 30% abnormal sperm heads and/or mid-piece defects, or more than 25% spermatozoa with proximal cytoplasmic droplets with reduced fertility.

In conclusions, the results of the present study concluded that NRR at 56 days after first insemination is related to parameters of semen quality in the fresh ejaculate. Hence, semen evaluation may allow the discarding of bulls with poor fertility from the AI program. Moreover, evaluation of semen before AI might be a good practice to increase NRR. Further study is needed to investigate the effects of other factors including cows' age, breed, parity and seasons of insemination performed and skillness of inseminator on NNR.

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