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MORPHOLOGICAL ABNORMALITIES OF FROZEN-THAWED BULL SPERM USED IN ARTIFICIAL INSEMINATION IN BANGLADESH

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ABSTRACT

Morphologically abnormal sperm reduces fertilization rates and embryonic development that results infertility. The objective of the study was to evaluate the morphological abnormalities of frozen-thawed bull sperm used for artificial insemination (AI) in Bangladesh. Isotonic formol saline fixed frozen-thawed sperm of local zebu (n=20) and crossbred (n=20) bulls were used in this study. Sperm morphology was determined for at least 200 sperm per bull by differential interference contrast (DIC) microscopy with oil immersion optics. Results showed that morphologically normal sperm were lower ($p<0.05$) in crossbred (74.6±1.8%) than zebu bulls (79.0±2.1%) whereas, abnormal sperms were higher in crossbred (25.5±1.8 %) than zebu bulls (21.0±2.1%). The specific sperm head defects were increased in crossbred than zebu bulls ($p<0.05$) but mid-piece and tail had no significant defects between crossbred and zebu bulls. Although morphologically abnormal sperms were higher in crossbred bulls, both types of bulls had standard satisfactory level of morphologically normal sperm *i.e* at least 70% morphologically normal sperm with no more than 20% of head abnormality. Therefore, the quality of frozen semen either from crossbred or zebu bulls distributed by the department of Livestock Services was found suitable to disseminate male germplasm for AI program in Bangladesh.

Keywords: Frozen-thawed sperm, bull, morphology, artificial insemination, Bangladesh

Introduction

Artificial insemination (AI) is one of the most important reproductive biotechnology tools that have made possible safe use of semen from selected sires in a breeding female population, thus preventing dissemination of venereal diseases (Thibier and Wagner 2000, Siddiqui *et al.* 2008). Furthermore, application of AI as a tool for dissemination of semen from sires with characters of importance has contributed to the improvement of the genetic quality of breeding herds. This improvement has been exponential in dairy cattle, in which use of frozen semen for AI is most common, providing possibilities for the commercial dissemination of genetic material all over the world (Kastelic and Thundathil 2008, Siddiqui *et al.* 2008). A prerequisite for the success of AI program is to use of the best quality semen from properly selected fertile bulls. For this reason, both screening of semen for normal morphology, and periodic evaluation of bull fertility are essential to the AI industry (Menon *et al.* 2011). AI of cattle in Bangladesh is increasing day by day (Siddiqui *et al.* 2008, 2013). Therefore, for improving the production and genetic merit of our non-descript indigenous cows, superior germplasm has been introduced all over Bangladesh (Sarder 2007, Siddiqui *et al.* 2013).

Bulls in commercial AI centers often have substantial variations in fertility, and frozen semen that meets minimum quality standards can yield pregnancy rates that differ by 20 to 25% (Larson and Miller 2000). Standard semen end points identify grossly abnormal semen, but do not consistently identify sub-fertile bulls with apparently normal semen (Kastelic and Thundathil 2008). As bull fertility is influenced by a wide range of factors, no single diagnostic test can accurately predict fertility (Petrunkina *et al.* 2007; Rodriguez-Martinez 2007). Moreover, the intrauterine deposition of semen in AI allows the use of small numbers of sperm (typically 20-25 million), so that the potential for abnormalities to affect fertility is increased. All the AI organizations, therefore, pay attention to sperm

morphology, although there is considerable variation in what is done and how it is interpreted (Menon *et al.* 2010).

Sperm morphology reflects the health of the seminiferous tubules and, to some degree the epididymis. The importance of sperm morphology in bull fertility has been well documented (Holroyd *et al.* 2002, Siddiqui *et al.* 2013). An understanding of the significance of specific types of sperm abnormalities in the ejaculate allows the diagnostician to make a prognosis of fertility and might indicate a course of treatment to assist in the recovery of a bull with abnormal sperm production (Siddiqui *et al.* 2008). Morphological abnormal sperm in semen has been associated with sub-fertility of bulls used in AI service (Menon *et al.* 2011). Spermatozoa with abnormal morphology are not uncommon even in semen collected from a good, proven fertility bulls, however the proportion of abnormal spermatozoa must remain within normal limit (Hafez and Hafez 2000). One of the simplest and reliable tests for the evaluation of infertility in bull is the estimation of different types of abnormalities in spermatozoa. In bull breeding soundness evaluation (BBSE), minimum standards include ≥70% morphologically normal sperm and ≤20% sperm with head defects (Barth and Oko 1989). Presence of 10% or more of any single type of head, mid-piece and tail and 20% or more of total abnormalities of spermatozoa is often coupled with reduced fertility in bull (Siddiqui *et al.* 2013). Al-Makhazoomi *et al.* (2008) reported that the relationship between sperm morphology and fertility after AI calls for frequent (2 to 3 month interval) detailed assessments of sperm morphology in AI stud bull.

In Bangladesh national breeding bull station, the information is available on the semen characteristics such as volume, color, density, mass activity, sperm concentration, motility, total number of sperm/ejaculate, total number of motile sperm/ejaculate, total number of semen doses/ejaculate and post-freezing motility of spermatozoa (Siddiqui *et al.* 2008). However, it is not known whether frozen-thawed zebu and cross-bred bull sperm are morphologically normal or abnormal that predict the success of AI program. Therefore, the present study was undertaken to evaluate the morphological abnormalities of frozen-thawed bull sperm used in AI in Bangladesh.

Materials and Methods

The study was carried out at Reproductive Biotechnology Laboratory, Department of Gynecology, Obstetrics and Reproductive Health, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. Collection, processing and morphological evaluation of frozen crossbred and zebu bull semen samples were performed during the period from July 2013 to June, 2014.

Source of frozen semen : The national herd of cattle in Bangladesh includes non-descript indigenous/local zebu and their crosses of Holstein-Friesian/Sahiwal sires. The frozen semen samples of zebu (n=20) and crossbred (n=20) bulls were collected for evaluation of sperm morphological abnormalities. The sperm samples were carried to the Reproductive Biotechnology laboratory, BSMRAU via liquid nitrogen (LN_2) container full of LN_2 and stored until processing and microscopic evaluation.

Morphological evaluation of frozen-thawed bull sperm: Sperm morphology was evaluated using a differential interference contrast (DIC) microscopy of wet-mount semen 'fixed' in isotonic formol saline solution at approximately 100x magnification. In order to avoid variations in morphology assessment between operations, all slides were evaluated by the same person. Two hundred spermatozoa were evaluated on each slide, focusing on the shape of sperm heads. However, as the identification of specific abnormalities was an important consideration, each defect was counted separately, even if multiple defects were occurred on the same sperm. All types of sperm morphological abnormalities were recorded, classified, and documented in record sheet (Table 1). The classification of sperm abnormalities was based on the findings during the microscopic evaluation.

Sperm abnormalities were designated as defects involving the head, mid-piece, or tail regarding more detailed characterization under the headings: abnormal head, detached head (decapitate), knobbed acrosome, proximal cytoplasmic droplet/pseudo-droplet (proximal droplet), distal cytoplasmic droplet/pseudo-droplet (distal droplet), translocated cytoplasmic droplet, double mid-piece, coiled tail, bent tail, folded tail, double tail, filamentous tail and others. Abnormal head included all deviations from the normal shape that were visually detectable including narrow base, pyriform, globular, asymmetrical contour, giant, undeveloped and broad/short (Table 1).

Statistical analysis : Descriptive statistics were performed using SPSS, version 17.5 (Chicago, USA).

Table 1. Bull Sperm morphological abnormalities record sheet with parameters

Bull ID:	
Normal Sperm	
Head & Acrosome defects	***Tail defects***
Double head	Double tail
Decapitated	Coiled tail
Pear shape	Simple bent tail
Narrow head	Reversed tail
Macrocephalic	Dag defect
Microcephalic	Stump tail
Pyriform	Folded tail
Narrow at base/ Tapered	Filamentous tail
Cratered	Broken/tail opening
Abnormal contour	
Ruffled acrosome	
Acrosome reacted	
Knobbed acrosome	
Mid-piece defects	
Swollen mid-piece	
Double mid-piece	
Proximal cytoplasmic droplets	
Distal cytoplasmic droplets	
Translocated cytoplasmic droplets	
Abaxial	
Corkscrew mid-piece	

Results and Discussion

In the present study, frozen semen samples from local zebu and crossbred bulls were procured and transported to the laboratory and processed for microscopic evaluation of sperm morphology. The representative morphological abnormalities of local zebu and crossbred bull frozen-thawed sperm are shown in Figure 1 (head abnormalities), Figure 2 (mid-piece abnormalities) and Figure 3 (tail abnormalities). Morphologically abnormal sperm can reduce rates of fertilization and embryonic development (Johnson 1997). It has been generally accepted that bull semen classified as satisfactory should contain ≥*70%* morphologically normal sperm with ≤*20%* sperm having abnormal head (Barth and Oko 1989, Kruger *et al.* 1988). The morphological sperm abnormalities of local zebu and crossbred bull semen are summarized in Table 2. Morphologically normal sperm were lower ($p<0.05$) in crossbred (74.6±1.8%) than local zebu bulls (79.0±2.1%); whereas, abnormal sperm were higher ($p<0.05$) in crossbred (25.5±1.8%) than local zebu bulls (21.0±2.1%). Many investigators have previously observed that sperm abnormalities is influenced by the breeds of bull (Sarder 2007, Menon *et al.* 2010) which is in agreement with the findings of the present study. The breeds of bull can influence the quality of preserved semen with regard to post-thaw motility and proportion of abnormal spermatozoa (Hallap *et al.* 2004, Menon *et al.* 2010). The breed-related variations in the semen quality can also be attributed to the differences in composition of seminal plasma (Siddiqui *et al.* 2013). Therefore, it is likely that the variation in the semen quality in the present study might be due to the differences in the age and breed of the bull.

The specific sperm head defects were increased in crossbred (11.4±0.8 vs. 8.8±0.7%) than local zebu bulls ($p<0.05$), but mid-piece (4.6±0.4 vs. 4.3±0.3%) and tail

Table 2. Sperm abnormalities (mean±SEM) of frozen bull semen used for AI

Breed	No. of bull	Sperm defects			Total	
		Head	Mid-piece	Tail	Abnormal	Normal
Local (L)	20	8.8[a]±0.7	4.3±0.6	8.0±1.0	21.0[a]±2.1	79.0[a]±2.1
Crossbred (H X S)	20	11.4[b]±0.8	4.6±0.4	9.7±1.0	25.5[b]±1.4	74.6[b]±1.8

Total 200 hundred spermatozoa were counted from each slide of each bull. Data were presented as mean±SEM; Values with different superscripts (a, b) within a column differed ($p<0.05$).

(9.7±1.0 vs. 8.0±1.0%) had no significant defects between crossbred and local zebu bulls, respectively. The specific sperm defects in the head (Table 3), mid-piece (Table 4) and tails (Table 5) are shown. The most common specific sperm defects were detached head or decapitate (1.9±0.3 vs. 1.3±0.3%), narrow at the base (1.7±0.3 vs. 1.4±0.2%), narrow head (1.5±0.3 vs. 1.5±0.3%), acrosome reacted (2.1±0.3 vs. 1.6±0.3%), proximal cytoplasmic droplets (1.9±0.2 vs. 1.8±0.4%), distal cytoplasmic droplets (1.0±0.2 vs. 1.4±0.2%), coiled tail (1.7±0.3 vs. 1.9±0.1%), bent tail (2.2±0.3 vs. 2.3±0.4%) and folded tail (2.5±0.4 vs. 1.9±0.4%) in both crossbred and local zebu bulls, respectively. In addition, other specific sperm defects recorded in the present study were macrocephalic, round head, pear shaped, cratered, knobbed acrosome (Figure 1), swollen mid-piece, translocated cytoplasmic droplets (Figure 2), double tail, filamentous tail (Figure 3).

Among the three major types of sperm defects (head, mid-piece and tail) in local zebu and crossbred bulls, tail defects were the most common followed by head and mid-piece. These results are partially consistent with the findings of a previous study (Menon *et al.* 2010), where authors found that mid-piece defects were the most common, followed by defects in the sperm head and tail. The differences in the present study might be due to the breed differences, age of bulls, climatic conditions, semen processing and so on. Among the detected various tail defects, folded tail was most predominant, followed by simple bent tail, coiled tail, stump tail, dag defects and broken tail

Table 3. Prevalence of major specific sperm head defects (Mean±SEM) of frozen bull semen used for AI

Breed	No. of bull	Head defects							
		DH	MIC	PI	NB	NH	AR	RA	Total
Local (L)	20	1.3[a]±0.3	0.5±0.2	0.8[a]±0.1	1.4±0.2	1.5±0.3	1.6[a] ± 0.3	0.6±0.1	8.8[a]± 0.7 (175)
Crossbred (H X S)	20	1.9[b]±0.3	0.3±0.2	1.4[b]±0.2	1.7±0.3	1.5±0.3	2.1[b]± 0.3	0.5±0.2	11.4[b]±0.8 (228)

DH- Detached head or decapitated; MIC- Microcephalic; PI- Piryform; NB-Narrow at the base; NH- Narrow head; AR- Acrosome reacted; RA- Ruffled Acrosome. Values with different superscripts (a, b) within a column differed ($p<0.05$).

Table 4. Prevalence of specific sperm mid-piece defects (Mean±SEM) of frozen bull semen used for AI

Breed	No. of bull	Midpiece defects						
		PCD	DCD	TCD	DM	Ab	CM	Total
Local (L)	20	1.8±0.4	1.4±0.2	0.5±0.1	0.0±0.0	0.8±0.3	0.2±0.1	4.3±0.6 (86)
Crossbred (H X S)	20	1.9±0.2	1.0±0.2	0.3±0.1	0.8±0.3	1.2±0.3	0.3±0.1	4.6±0.4 (92)

PCD- Proximal Cytoplasmic Droplets; DCD- Distal Cytoplasmic Droplets; TCD- Translocated Cytoplasmic Droplets; DM- Duplicated mid-piece; Ab-Abaxial; CM-Corkscrew Mid-piece. Values with different superscripts (a, b) within a column differed ($p<0.05$).

Table 5. Prevalence of specific sperm tail defects (Mean±SEM) of frozen bull semen used for AI

Breed	No. bulls	Tail defects						
		CT	BT	DD	ST	FT	TO	Total
Local (L)	20	1.9±0.1	2.3±0.4	0.3±0.1	0.7±0.2	1.9[a]±0.4	0.4±0.2	8.0±1.0 (159)
Crossbred (H X S)	20	1.7±0.3	2.2±0.3	0.7±0.1	1.1±0.2	2.5[b]±0.4	0.6±0.2	9.7±1.0 (193)

CT- Coiled tail; BT- Bent tail; DD- Dag defects; ST- Stump tail; FT- Folded tail; TO- Broken/Tail opening. Values with different superscripts (a, b) within a column differed ($p<0.05$).

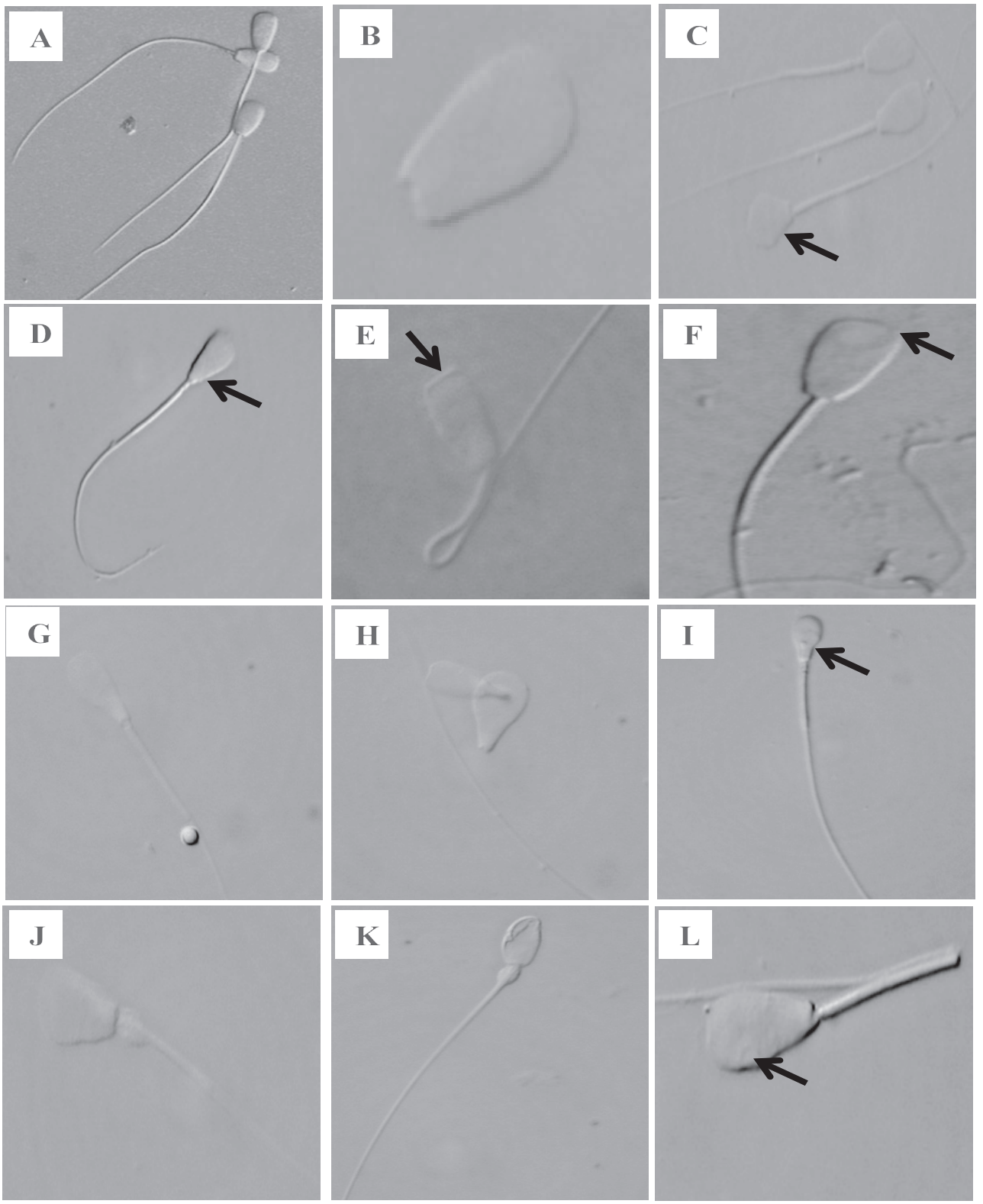

Figure 1. Photomicrographs of normal and head defects frozen-thawed bull sperm used for AI in Bangladesh. (DIC microscopy, 100x) A. Normal sperm, B. Decapitated, C. Round head, D. Narrow at the base or tapered, E. Knobbed or Acrosome reacted, F. Ruffled acrosome, G. Narrow head, H. Pyriform and decapitated, I. Pyriform, J. Macrocephalic and proximal cytoplasmic droplets, K. Microcephalic , acrosome reacted and proximal cytoplasmic droplets, L. Diadem defect or cratered.

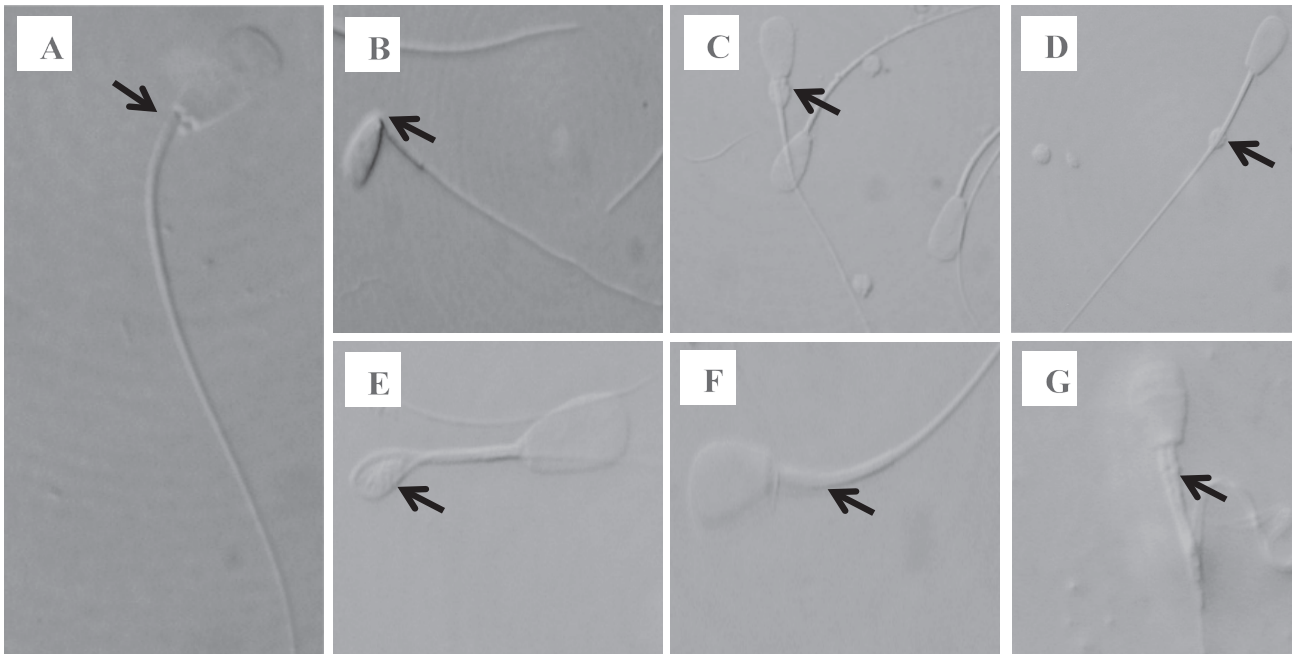

Figure 2. Photomicrographs of mid-piece defects frozen-thawed bull sperm used for AI in Bangladesh. (DIC microscopy, 100x) A. Abaxial, B. Broken mid-piece, C. Proximal cytoplasmic droplets, D. Distal cytoplasmic droplets, E. Translocated cytoplasmic droplets, F. Swollen mid-piece, G. Double mid-pieces and double tails.

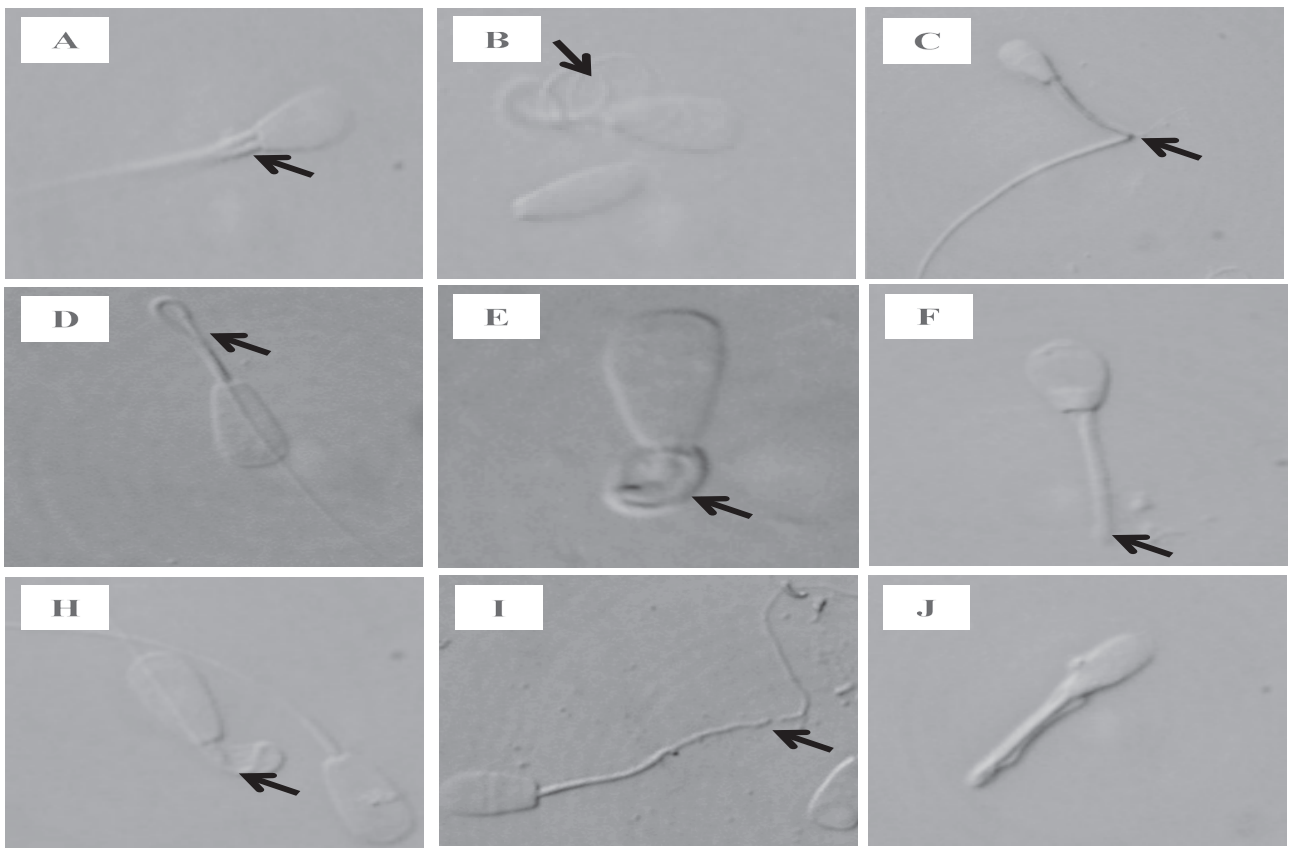

Figure 3. Photomicrographs of tail and droplets defect frozen-thawed bull sperm used for AI in Bangladesh. (DIC microscopy, 1000x), A. Double tail, B. Coiled tail, C. Simple bent tail, D. Reversed tail, F. Dag defect, G. Stump tail, H. Folded tail, I. Filamentous tail, J. Broken/tail opening.

defects. This observation was consistent with an earlier report that folded tail is the most common sperm tail abnormality found in bull semen, regardless of breed (Barth and Oku 1989). Although this defect had little effect upon fertility in AI, it caused subfertility in natural service sires (Johnson 1997). Of the head abnormalities encountered, detached head and acrosome reacted were most common. In bull semen with acceptable sperm quality, it was common to find a small percentage of detached sperm heads. Similarly, an average prevalence of 5.3% detached heads was found in western Canadian range bulls (Barth and oku 1989). The common causes for large number of sperm with detached heads in a semen sample are testicular hypoplasia, testicular degeneration, and senescence of sperm due to sexual inactivity. Among the various mid-piece defects detected, proximal mid-piece droplets was most predominant, followed by distal mid-piece droplets defect. This observation was consistent with a previous report that proximal and distal mid-piece droplets defects are most common sperm tail abnormality found in bull semen, regardless of breed (Barth and oku 1989, Siddiqui *et al.* 2008). Although this defect had little effect upon fertility in AI, it caused sub-fertility in natural service sires (Soderquist *et al.* 1991, Johnson 1997).

Conclusion

The morphological abnormalities of frozen-thawed sperm were lower in zebu than crossbred bulls used in AI program in Bangladesh. However, both types of bull had a standard level of morphological normal sperm. Therefore, the findings of this study suggest that frozen semen either from crossbred or zebu bulls can be used in a successful AI program if sperm morphology maintained at least ≥70% morphologically normal sperm with ≤20% head abnormality. Further studies are needed to assess the fertility rate after AI of frozen semen and evaluate the success of AI program in Bangladesh.

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