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MORPHOLOGICAL SPERM ABNORMALITIES OF DIFFERENT BREEDS OF AI BULL AND ITS IMPACT ON CONCEPTION RATE OF COWS IN AI PROGRAMME

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ABSTRACT

The main objective of this study was to evaluate the effect of genetic groups of bulls on morphological characteristics of spermatozoa in relation to conception rate of first inseminated cows. For this purpose, 1390 ejaculates of semen were collected from 71 bull of six genetic groups at Central Cattle Breeding Station and Dairy Farm (CCBSDF), Savar, Dhaka; Rajabarihat Dairy Cattle Improvement Farm (RDCIF), Rajshahi, and District Artificial Insemination Centre, Rajshahi, from January 1997 to March 2002. A total of 20936 cows were inseminated by 40 technicians of different AI Sub-centre or points under the District Artificial Insemination Centre, Rajshahi, and pregnancy was confirmed on rectal palpation at 90 to 120 days after insemination. The average values of total head abnormalities, free loose head, mid-piece, tail abnormalities, proximal and distal cytoplasmic droplets, total tail abnormalities and total sperm abnormalities were 5.45%, 2.44%, 1.19%, 6.19%, 0.74%, 0.57%, 11.16% and 16.38%, respectively. Genetic groups of bull had significant ($p < 0.05$) effect on all the morphological characteristics of spermatozoa. The lower incidence of total sperm abnormalities (13.45%) was found in the genetic groups of 100% SL and higher (19.28%) in 75% F × 25% L bulls. The genetic groups of bulls had significant effect on first service conception rate of cows. The conception rate was higher in cows inseminated with 100% Local than those inseminated with genetic groups of 75% F × 25% L (53.13% vs 45.88%; $p < 0.05$). Significantly strong positive correlations were found between total head abnormalities and total sperm abnormalities ($r = 0.828$; $p < 0.01$) as well as between total tail abnormalities and total sperm abnormalities ($r = 0.892$; $p < 0.01$). It appears from the results that minimum total sperm abnormalities are found for pure breed bulls than cross-bred bulls, and 100% Local, 100% Friesian and 50% SL × 50% F bulls had better conception rates on cows.

Key words: Spermatozoa, abnormalities, bull, genetic group, AI, conception rate

INTRODUCTION

Morphologically abnormal spermatozoa are unable to fertilize the oocytes (Shamsuddin and Rodriguez-Martinez, 1994). 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 a normal limit (Hafez, 1999). The fertility of the spermatozoa is directly correlated with the percent motility of spermatozoa. Salisbury *et al.* (1978) recommended not to tolerate >20% abnormalities of sperm head and / or mid piece in routine AI practice. One of the simplest and reliable tests for evaluation of infertility in bulls is the estimation of different types of abnormalities in spermatozoa. 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 (Hancock, 1955). Reddy *et al.* (1975) have reported significant negative correlation between abnormal spermatozoa and conception rate. Total sperm abnormalities were positively significant influenced by abnormalities at head (0.650) and mid-piece (0.723) regions (Hazarika *et al.*, 1988). The bulls used for artificial insemination in Bangladesh are mostly crosses between *Bos indicus* and *Bos taurus*. The adaptability of such cross-bred bull in hot and humid climate as prevailing in Bangladesh is not without question. The present investigation was undertaken to evaluate the effect of genetic groups of AI bulls on incidence of abnormalities of spermatozoa and in relation to conception rate of inseminated cows, and to examine the correlation between the different sperm abnormalities.

MATERIALS AND METHODS

The study was conducted on morphological abnormalities of spermatozoa in different breeds of 71 AI bulls at three AI centres / stations (Central Cattle Breeding Station and Dairy Farm, Savar, Dhaka; Rajabarihat Dairy Cattle Improvement Farm, Rajshahi, and District Artificial Insemination Centre, Rajshahi) and 40 AI Sub-centres / Points on 20,936 cows for first insemination under District AI centre, Rajshahi during the period from January 1997 to March 2002. The experimental AI bulls were divided into 6 (six) groups (A to F) according to their genetic composition; Group A = 100% Friesian (100% F, n = 11), Group B = 100% Sahiwal (100% SL, n = 11), Group C = ≥ 75% Friesian × ≤ 25 % Local (≥ 75% F × ≤ 25 % L, n = 14), Group D = ≥ 50% Sahiwal × ≤ 50% Friesian (50% SL × 50% F, n = 11), Group E = 50% Friesian × 50% Local (50% F × 50% L, n = 14) and Group F = 100% Local (100% L, n = 10). A total of 676 ejaculates of semen for tail abnormities and 714 ejaculates of semen for head abnormalities from 71 AI bulls were obtained over a period of 5 years considered as the basic materials for the study. The following support chemical reagents viz. basic fucshin, alcohol, phenol, bluish eosin, chloramine for William's stain and disodium hydrophosphate, potassium di-hydrogen phosphate, sodium chloride and formaldehyde for formal saline used as fixative were used.

The bulls of three centres / stations were regarded as clinically healthy and free from any significant abnormalities. The bulls received routine vaccination against anthrax, haemorrhagic septicemia, blackleg, foot and mouth disease and anthelmintics against fascioliasis and round worm infections. Bulls were tested for fertility before putting them in the breeding herd. The feeding and management systems of bulls were more or less same but environmental condition was different.

The bulls were trained to ejaculate in artificial vagina (AV) at homosexual mount using at three AI centres. After collection, semen samples were kept at 35°C in water-bath until the media and reagents were added to the samples. Semen was collected from bulls generally interval once in a week of all the AI centres. After routine semen examination and two types of semen (chilled semen and frozen semen) were produced for AI.

For chilling, the semen was diluted with egg yolk-citrate extender at a rate of 20×10^6 actively motile spermatozoa in an insemination dose of 1 ml. The diluted semen was preserved in aliquots (1 ml) at 4 to 8 °C until used. Only the semen showing ≥ 50% motility after processing was selected for AI. For freezing semen, CCBSDF, Savar, and RDCIF, Rajshahi, routinely used egg yolk-tris-fructose-citric acid-glycerol extender. The final concentration of spermatozoa was 30×10^6 / 0.25 ml. According to the AI centres, only semen that showed ≥ 40% post-thaw motility was adopted for AI.

Morphological examination was done by staining the smear of semen with William's method (Williams, 1920). A thin smear of fresh semen was prepared on a grease free slide for the study of morphological abnormalities of sperm head after staining with William's stain. The smear was air dried and fixed in flame and some information like bull ID and date of semen collection were marked on slide at any end with the help of permanent marker pen. Then the smear was treated with absolute alcohol for 3-4 minutes and washed with 0.5% chloramine for 1-2 minutes until it appeared fairly clear and then washed in distilled water followed by rinsing in 95% alcohol and finally stained with Carbol-fuchsin eosin for 8-10 minutes. After staining, the slides were washed in running tap water, then dried off and examined under light microscope with oil emersion at 1000X. The proportion of sperm with normal head morphology included only those were free from any detectable abnormalities. The head abnormalities in spermatozoa (Fig. 1) were classified as per description of Williams (1920) such as pear shape head, narrow at the base head, narrow head, broad, big and little short head, abaxial position of the mid piece, undeveloped head, abnormal contour and others abnormal head. At least 500 spermatozoa from individual smears were examined. The proportion of spermatozoa with abnormal head morphology included only those, which were any detectable abnormalities.

The morphology of sperm mid-piece and tail was studied after fixed with buffered formol saline at the same temperature. Buffer-formol saline was prepared according to the method described by Hancock (1957). One ml of formol saline was taken in sample glass tube with plastic cork and a very small drop or 10 µl of fresh semen was mixed, shaked and marked the sample with the information like bull ID, breed and date of semen collection for later examination and sample was preserved for a long time in refrigerator (+ 4°C). The abnormalities of formol saline fixed spermatozoa were observed under phase contrast microscope. The following abnormalities were found in the freshly collected and preserved semen viz. free loose head, abnormal mid-piece, abnormal tail (simple bent tail and coil tail), proximal and distal cytoplasmic droplets and double folded tail, broken neck, abnormal acrosome and others. At least 200 spermatozoa from individual replicates were examined at 1000X magnification. The proportion of abnormal spermatozoa at formol saline preparation included only those, which had abnormalities in the mid-piece and tail. The spermatozoa were considered as normal having no mid-piece and tail defects (Fig. 2).

For evaluation of the fertility, semen was distributed to 40 AI technicians randomly at different AI Sub Centres / Points under the District AI Centre, Rajshahi. AI was performed in the study areas by 40 AI technicians. All AI technicians received a one year training on AI and are involved in routine AI activities covering 90 to 450 AI monthly. The cows to be inseminated were detected in oestrus by the farmers based upon the clinical and behavioral signs and were delivered to AI centres or points. However, the cows in urban areas were often inseminated in the farm. The cows were inseminated transcervically by recto-vaginal method (Salisbury *et al.*, 1978) with the help of inseminating gun in case of frozen semen or plastic AI tube / pipette for chilled semen and cows were inseminated by inseminators at about 12-18 h of oestrus. The pregnancy was confirmed on rectal palpation of the genital tract at 90 to 120 days after insemination (Morrow, 1986). The data were calculated for the first service conception rate. The conception rate (CR) was calculated by using the following formula:

$$\text{C.R (\%)} = \frac{\text{No. of animals conceived}}{\text{No. of animals inseminated and examined}} \times 100$$

Statistical analysis

Mean and standard deviation in sperm abnormalities of tail and head as well as conception rate of inseminated cows among the genetic groups of AI bulls were calculated using the computer programme SPSS (Anon., 1996). For categorical data, the means were compared by using Duncan's Multiple Range Test (Steel and Torrie, 1980). The correlation coefficient among the sperm abnormalities was calculated by using Correlate-Bivariate (Pearson two tailed) analysis.

RESULTS AND DISCUSSION

In total 1390 ejaculates of semen from 71 bulls of six genetic groups collected over a period of 5 years and were studied for different types of sperm abnormalities in head, mid-piece and tail regions in relation to conception rate of first inseminated cows. Significant differences were found among the genetic groups of AI bulls in Artificial Insemination (AI) centres / stations in head, mid-piece and tail of the spermatozoa. The percent abnormalities in head, mid-piece and tail of spermatozoa of six genetic groups of bulls in relation to conception rate of inseminated cows in AI programme are shown in Table 1.

Table 1. Effect of genetic groups of bulls on the morphological abnormalities of spermatozoa in relation to conception rate of inseminated cows in AI programme

Parameters	Genetic groups of AI bulls						Grand total
	100% F (n = 11)	100% SL (n = 11)	75% F-25% L (n = 14)	50% SL-50% F (n = 11)	50%F-50% L (n = 14)	100% L (n = 10)	
Head abnormalities (%)	**Ne = 107**	**Ne = 112**	**Ne = 155**	**Ne = 108**	**Ne = 137**	**Ne = 95**	**Ne = 714**
Pear shape head	0.80±0.6^{a}	0.91±0.4^{a}	0.87±0.6^{a}	0.93±0.1^{a}	0.80±0.6^{a}	0.35±0.2^{b}	0.79±0.6
Narrow at base head	1.76±0.7^{b}	1.44±0.8^{c}	2.00±0.8^{a}	1.75±1.3^{b}	1.76±0.5^{b}	1.34±0.6^{c}	1.71±0.8
Narrow head	0.97±0.8^{b}	0.87±0.5^{b}	1.14±0.8^{a}	0.90±0.6^{b}	1.13±0.6^{a}	1.11±0.7^{a}	1.03±0.7
Undeveloped head	7.327 E-02 ±0.1^{c}	0.11±0.2^{bc}	0.21±0.41^{a}	0.16±0.3^{ab}	0.14±0.3^{abc}	8.989E-02 ±0.1^{bc}	0.13±0.3
Big, little and broad head	0.99±0.4^{c}	0.50±0.5^{d}	1.45±0.7^{a}	0.92±0.8^{c}	1.26±0.6^{b}	1.04±0.7^{c}	1.06±0.7
Abaxial head	0.11±0.1^{bc}	0.12±0.2^{bc}	0.28±0.3^{a}	6.620E-02 ±9.923E-02^{c}	0.16±0.2^{bc}	0.11±0.22^{bc}	0.15±0.2
Others	0.61±0.4^{ab}	0.33±0.2^{c}	0.68±0.6^{a}	0.31±0.6^{c}	0.74±0.5^{a}	0.48±0.5^{b}	0.54±0.5
Total head abnormalities (%)	**5.34±1.3^{c}**	**4.31±1.4^{d}**	**6.66±1.8^{a}**	**5.06±2.6^{c}**	**6.03±1.5^{b}**	**4.54±1.1^{d}**	**5.45±1.9**
Tail abnormalities (%)	**Ne = 117**	**Ne = 98**	**Ne = 140**	**Ne = 96**	**Ne = 130**	**Ne = 95**	**Ne = 676**
Free loose head	2.41±0.9^{c}	2.19±0.6^{d}	2.62±0.7^{ab}	1.95±0.5^{e}	2.55±0.7^{bc}	2.80±0.5^{a}	2.44±0.7
Mid-piece abnormalities	1.31±0.6^{a}	1.10±0.4^{b}	1.18±0.4^{ab}	1.12±0.5^{b}	1.35±1.1^{a}	1.02±0.4^{b}	1.19±0.6
Tail abnormalities	6.20±1.7^{c}	5.04±1.6^{d}	7.25±1.3^{a}	6.47±1.5^{bc}	6.61±1.1^{b}	4.97±1.1^{d}	6.19±1.6
Proximal cytoplasmic droplets	0.75±0.6^{a}	0.59±0.3^{b}	0.85±0.4^{a}	0.77±0.5^{a}	0.73±0.4^{ab}	0.74±0.4^{a}	0.74±0.8
Distal cytoplasmic Droplets	0.65±0.4^{a}	0.45±0.3^{b}	0.76±0.4^{a}	0.53±0.4^{b}	0.53±0.4^{b}	0.40±0.2^{c}	0.57±0.4
Total tail abnormalities (%)	**11.35±2.2^{b}**	**9.38±1.9^{e}**	**12.68±1.5^{a}**	**10.86±1.9^{c}**	**11.78±1.9^{b}**	**9.94±1.2^{d}**	**11.16±2.1**
Total sperm abnormalities (%)	**16.40±2.7^{c}**	**13.45±2.8^{e}**	**19.28±2.5^{a}**	**15.13±2.9^{d}**	**17.38±2.7^{b}**	**14.69±1.9^{d}**	**16.38±3.2**
No. of animals inseminated	3009	452	4196	2798	5227	1184	20936
No. of animals conceived	1570	279	1925	1469	2637	629	10509
Conception rate (%)	52.18^{ab}	50.40^{b}	45.88^{c}	52.50^{ab}	50.45^{b}	53.13^{a}	50.20

F = Friesian, SL = Sahiwal, L = Local (non descript), n = No. of bulls, Ne = No. of ejaculates examined, Means having different superscripts in a same raw differed significantly ($p < 0.05$).

The mean values of different sperm abnormalities obtained in this study were : total head abnormalities 5.45%, free loose head 2.44%, mid-piece abnormalities 1.19%, tail abnormalities 6.19%, proximal cytoplasmic droplets 0.74%, distal cytoplasmic droplets 0.57%, total tail abnormalities 11.16% and total sperm abnormalities 16.38% among the six genetic groups of bulls (Table 1). Genetic groups of bull had significant ($p < 0.05$) effect on all the morphological characteristics of spermatozoa (Table 1). The incidence of total sperm abnormalities was significantly ($p < 0.05$) high (19.28%) in 75% F × 25% L and low in the genetic group of 100% SL (13.45%). Significantly ($p < 0.05$) highest total sperm abnormalities was found in cross-bred bull than those of pure breeds.

The conception rate was significantly ($p < 0.05$) higher in cows inseminated with 100% Local (53.13%) than those inseminated with 75% Friesian × 25% Local (45.88%) bulls. There were no significant differences in conception rate of cows inseminated with semen of 100% Friesian (52.18%) and 50% SL × 50% F (52.50%) bulls.

Correlation coefficients among the percentages of abnormalities of spermatozoa are shown in Table 2. Most of the abnormalities of spermatozoa were found to have significant positive correlation. Pear shape head and narrow head were found to be negatively correlated (r = -0.023), and free loose head and distal cytoplasmic droplet were also negatively correlated (r = -0.134). Significantly strong positive correlations were found between total head abnormalities and total sperm abnormalities (r = 0.828) as well as between total tail abnormalities and total sperm abnormalities (r = 0.892) (Table 2).

Table 2. Correlation between various abnormalities (%) of spermatozoa of bulls used for AI programme

S/N	Sperm abnormalities	1	2	3	4	5	6	7	8	9	10	11	12
1.	Pear shape	1.00											
2.	Narrow at base	.113	1.00										
3.	Narrow head	-.023	.282*	1.00									
4.	Big, little and broad	.009	.093*	-.048	1.00								
5.	Total head abnormalities	.366*	.640**	.501**	.499**	1.00							
6.	Free loose head	.001	.166	.199**	.207*	.315*	1.00						
7.	Mid-piece	.050	.102	.024	.050	.154	-.023	1.00					
8.	Tail abnormalities	.032	.69**	.106*	.362*	.394*	.204*	-.008	1.00				
9.	Proximal cytoplasmic droplets	.088	.030	.059	.003	.053	-.099	- .033	-.021	1.00			
10.	Distal cytoplasmic droplets	.059	.065	-.035	-.011	.016	-.134	-.047	.005	.031	1.00		
11.	Total tail abnormalities	.075	.322*	.167	.369*	.485**	.471**	.319*	.839**	.186	.154	1.00	
12.	Total sperm abnormalities	.237*	.538**	.366*	.492**	.828**	.465**	.284*	.742**	.147	.107*	.892**	1.00

*Correlation is significant at $p < 0.05$ level (2 tailed), **Correlation is significant at $p < 0.01$ level (2 tailed).

Many investigators observed the incidence of spermatozoa with abnormal morphology in fertile bulls to be 0-18% which agree the findings of present study (13.45-19.28%) whereas Zemjanis (1970) reported the incidence of total spermatozoa abnormalities in the semen of fertile bulls to be 30-40%. Individual bulls differed with respect to head abnormalities, free loose head abnormalities, mid-piece abnormalities, tail abnormalities, proximal cytoplasmic droplet and distal cytoplasmic droplets and total sperm abnormalities. The differences among bulls may be due to variation in their scrotal circumference, breed, age, body weight and body size and the secretory activities of their sex glands (Leon *et al.*, 1991; Sharma *et al.*, 1991). Moreover, collection frequency, precollection sexual stimulation, feeding regimen and climatic conditions can also influence the sperm abnormalities (Al-Hakim *et al.*, 1986). Breeds of bull had significant ($p < 0.05$) effect on sperm abnormalities. The average total percentages of head abnormalities in 100% F, 100% SL, 75% F x 25% L, 50% SL x 50% F, 50% F x 50% L and 100% L bulls were found to be 5.34, 4.31, 6.66, 5.06, 6.03 and 4.54%, respectively. The values recorded in the present study for six breeds are lower than the average values reported in the literature for the normal fertile bulls (Rao and Rao, 1975). The average spermatozoa of loose head in 100% F, 100% SL, 75% F × 25% L, 50% SL × 50% F, 50% F × 50% L and 100% L bulls were observed to be 2.41, 2.19, 2.62, 1.95, 2.55 and 2.80 respectively which support the reports of Rao and Rao (1975). The occurrence of loose head was found to be significantly ($p < 0.05$) higher in 100% L bulls than in the 100% SL bulls. The average percentage of the mid-piece abnormalities was found to be ranged 1.02-1.35% among genetic groups of bulls, which is in conformity with the reports of Rao and Rao (1975). The average percentages of tail abnormalities were found to be 6.20, 5.04, 7.25, 6.47, 6.61 and 4.97, respectively for 100% F, 100% SL, 75% F × 25% L, 50% SL × 50% F, 50% F × 50% L and 100% L. Proximal cytoplasmic droplet was found to be 0.74%.

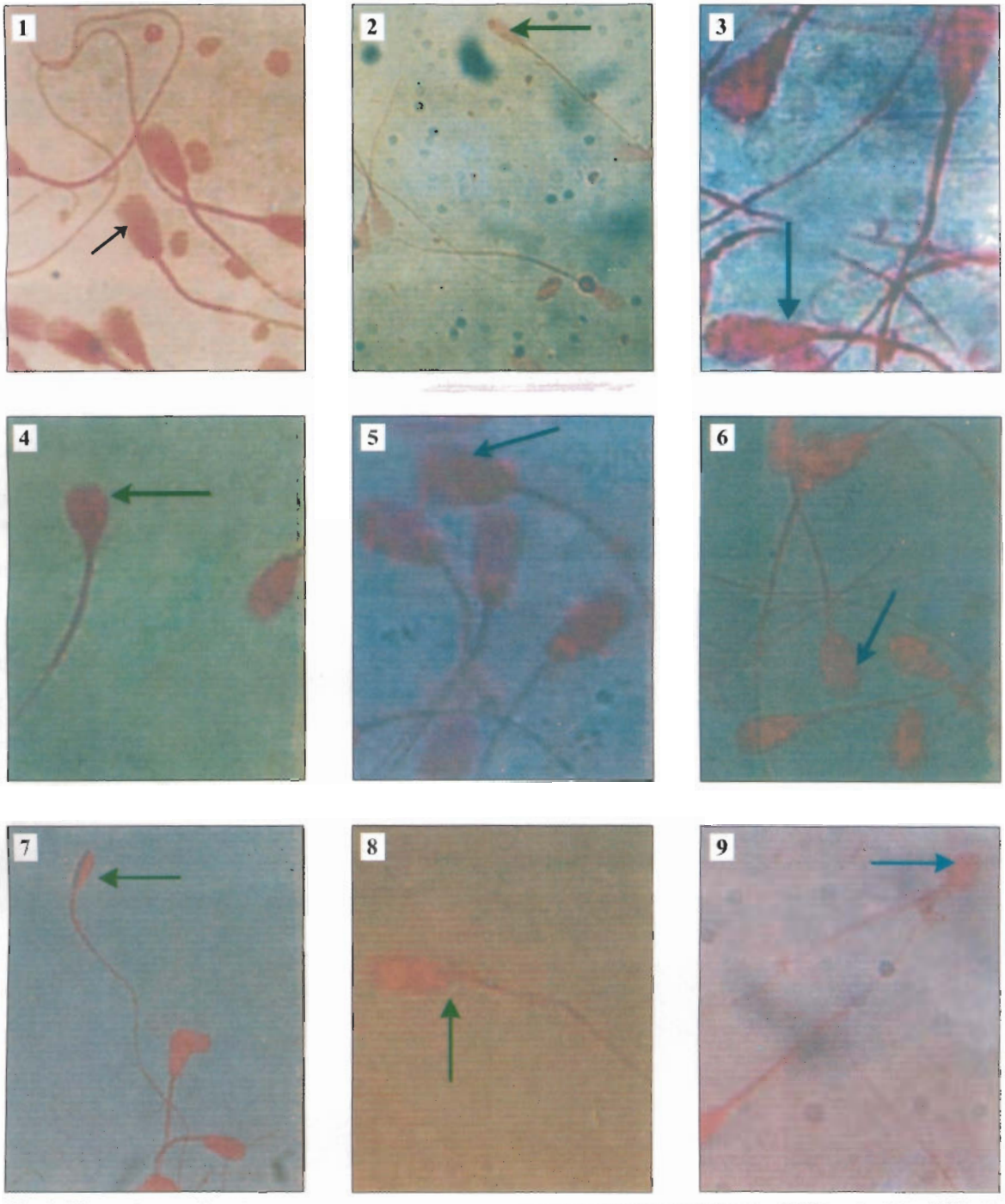

Fig. 1. Photomicrographs of normal and abnormal head morphology of bull spermatozoa (William's stain 1000x). 1: Normal head, 2: Narrow head, 3: Pear-shaped head, 4: Narrow at base of a sperm head, 5: Big head, 6: Broad head, 7: Undeveloped head, 8: Abaxial connection between head and neck and 9: Round sperm head.

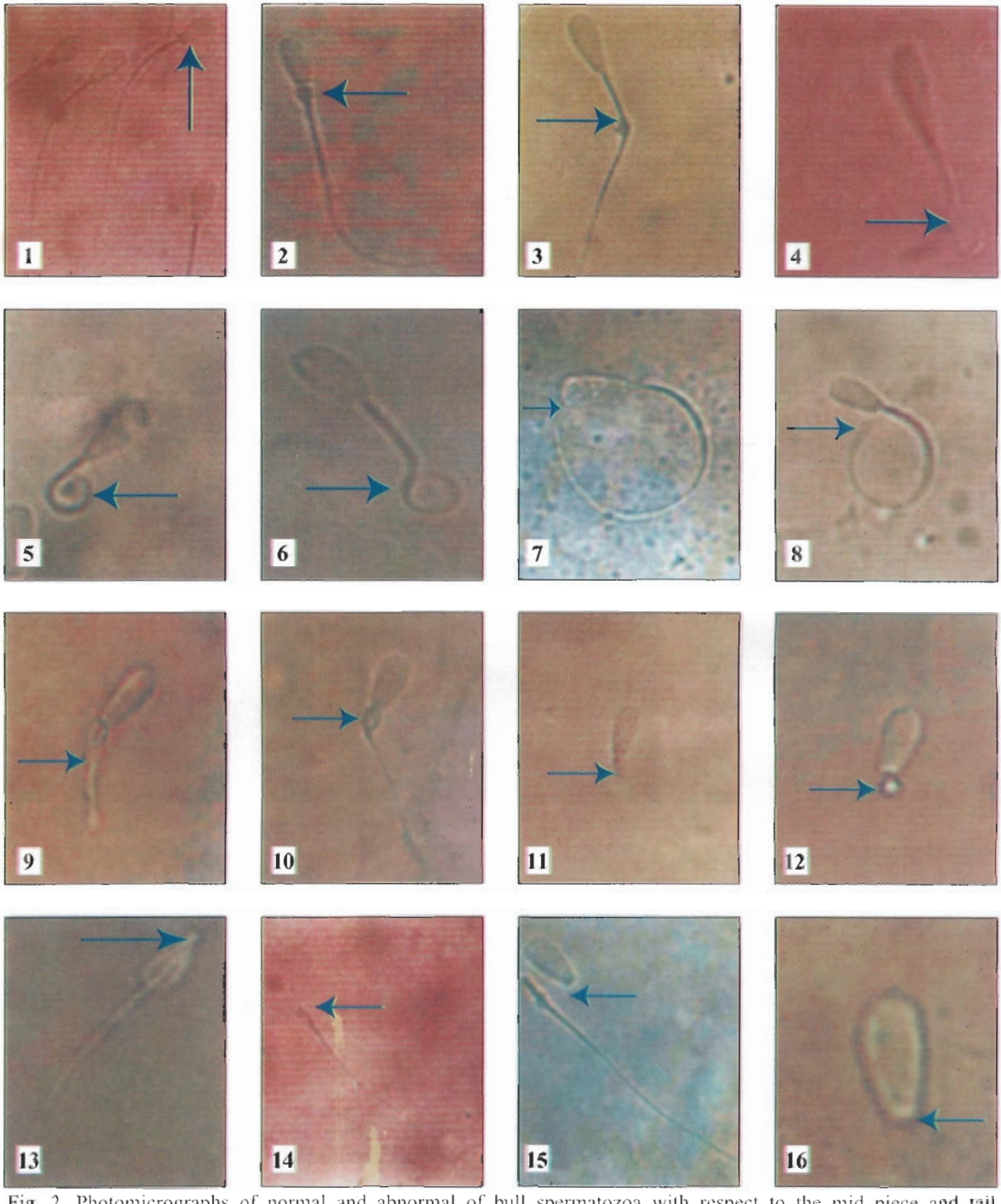

Fig. 2. Photomicrographs of normal and abnormal of bull spermatozoa with respect to the mid piece and tail (Phase contrast optics,1000x). 1: Normal spermatozoa, 2: Proximal cytoplasmic droplet, 3: Distal cytoplasmic droplet, 4: Simple bent tail, 5: Coil tail, 6: Coil tail in lower part of tail, 7: Tail coil around the head of the spermatozoa, 8: Tail coil below the head of the spermatozoa, 9: Double folded tail, 10: Mid-piece defect, 11: Mid-piece and tail defect, 12: Tailless mid-piece, 13: Tail broken at the neck of the spermatozoa, 14: Abnormal acrosome, 15: Sperm head detached from mid-piece and 16: Free loose head.

Highly significant breed variations in the occurrence of sperm abnormalities in six genetic groups of bulls have been recorded in the present study. The normal spermatozoa in respect of mid piece and tail abnormalities was lower in 50% F × 50% L and 75% F × 25% L groups than the other genetic groups. The mean abnormal spermatozoa with head morphology was significantly ($p < 0.05$) higher (6.66%) in 75% F × 25% L than the other genetic groups of bulls. Tegegne *et al.* (1994) recorded higher proportion of normal spermatozoa in Boran than in Boran × Friesian bulls in Ethiopia. Again, Bhupal *et al.* (1993) observed the better motility of spermatozoa of Holstein bull than that of Sahiwal bulls which differs with the present findings. The variation may be resulted from differences in composition of seminal plasma in different breeds of animals due to their genetic origin (Nandroo *et al.*, 1987; Mohan *et al.*, 1994).

The breed of the bull can influence the quality of preserved semen with regard to post-thaw motility and proportion of abnormal spermatozoa (Bhupal *et al.*, 1993; Tegegne *et al.*, 1994). The breed-related variation in the semen quality can also be attributed to the differences in composition of seminal plasma (Nandroo *et al.*, 1987; Mohan *et al.*, 1994). Therefore, it is likely that the variation in the semen quality in the present study may be due to differences in the age and breed of the bull. The proportion of morphologically abnormal spermatozoa in semen correlates negatively with fertility results (Shamsuddin *et al.*, 1993; Shamsuddin and Rodriguez-Martinez, 1994). *In vitro* studies proved that morphologically abnormal spermatozoa failed to pass through the hyaluronic acid of the female genital tract to reach and penetrate the oocytes (Shamsuddin and Rodriguez-Martinez, 1994). The variations in the conception rate among the genetic groups of bulls in the present investigation may be due to fact that the best quality bull semen contained higher proportion of morphologically normal motile spermatozoa than that of poor bull semen. It is well documented that the fertilizing capacity of spermatozoa depends on the innate fertilizing capacity of bulls as well as deposition of optimum number of morphologically normal spermatozoa into the uterus in time.

It can be concluded from the present study that the incidence of abnormal spermatozoa was comparatively lower in genetic groups of 100% Sahiwal and 100% Local as well as 100% Local, 50% SL × 50% F and 100% Friesian bulls were suitable for the higher conception rate of cows in AI programme.

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