COMPARATIVE STUDY ON SPERM MORPHOLOGY AND MORPHOMETRY OF HOLSTEIN FRIESIAN AND MURRAH BUFFALO BULL

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ABSTRACT

The aim of this study was to compare the morphometry of the entire sperm of Holstein Friesian and Murrah buffalo bull and also to compare various staining techniques for the evaluation of sperm morphology by light microscopy. For this research work, twelve semen samples, six from Holstein Friesian and six from Murrah buffalo were collected. Smears of neat semen were stained with Hematoxylin Eosin, Toluidine Blue, Silver Nitrate and Eosin Nigrosin dyes. Seven measurements were made including the head length, head width, head base length, acrosomal cap length, acrosomal cap width, mid piece length and total tail length for two hundred spermatozoa with normal morphology. In the present study the mature spermatozoon consisted of head, neck, middle piece, principal piece and end piece. These morphometric measurements were almost similar for the various stains used. In addition, it indicate that Haematoxylin Eosin and Eosin Nigrosin are the suitable staining methods for evaluation of sperm head, Toluidine Blue is suitable stain for the evaluation of mid piece and Eosin Nigrosin is a effective stain for the evaluation of acrosomal cap. Appreciable evaluation was not possible in smears stained with Silver nitrate. Holstein Friesian sperm was longer in length than Murrah buffalo sperm length. The measurements for head length, head width, mid piece length and total tail length were significant ($P<0.01$) whereas the measurements for head base length, acrosomal cap length and acrosomal cap width were non significant ($P<0.01$).

Keywords: *Bubalus bubalis*, buffalo, sperm morphology, sperm morphometry, Holstein Friesian, Murrah buffalo

INTRODUCTION

In India, buffalo and cattle are important livestock resources, providing milk, meat and draught power in many ecologically disadvantaged agricultural systems, so it is being the mainstay in rural economy contributing dairy and meat industries. The best dairy breed in India among exotic cattle and buffalo breed regarding milk production is Holstein Friesian and Murrah respectively. Hence, cattle and buffalo are major genetic resources, contributing in a big way towards agricultural Gross domestic product. Therefore, development and application of suitable reproductive technologies can be expected to lead to a thrust in dairy and meat industry. There is hardly

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any information forthcoming for morphology of exotic bulls and buffalo bulls. So a modest has been made here to study the seminal attributes and morphology of Holstein Friesian (HF) cattle bulls and Murrah buffalo bulls.

Around three hundred years ago, Leeuwenhoek using a simple light microscope described the sperm as a kind of animalcule. Since this time, great advancements in light microscopy provided the impetus for continuous study of sperm morphology (Kojima and Yoshio, 1966). Sperm morphology, motility and concentration are considered to be the three most important parameters when assessing the semen quality. Morphological features of sperm serve as a reliable indicator in predicting the fertilizing capacity of sperm and also reflect certain disorders of spermatogenesis

The shape and size of spermatozoa are species- specific and differ between the animals such as cattle (Boersma *et al*., 2001; Beletti *et al*., 2005), sheep (Gravance *et al*., 1998; Sancho *et al*., 1998), goat (Hidalgo *et al*., 2006) and horse (Arruda *et al*., 2002). Existence of significant differences in sperm morphology and morphometry between species suggests that dimensional characteristics of spermatozoa are genetically controlled. Normal sperm morphology may be an indicator of the fertility potential of a male (Padrik and Jaakma, 2002; Esteso *et al*., 2006). Morphology of spermatozoa is an important for a successful fertilization and early embryonic development in assisted reproductive techniques (Kuster *et al*., 2004).

Morphometric analysis of sperm heads has been shown to be an indicator *in vitro* fertility (Thompson *et al*., 1994). Sperm morphometry, in combination with other objective traits, can be useful for developing a fertility index. In the dairy industry, where artificial insemination is the standard, male fertility is defined as the percentage of females inseminated that are not re-inseminated a defined number of days after the first insemination (Kumar and Krupakaran, 2014).

Manual methods of analysis are subjective, and highly variable within and between technicians, which may account for these differences. Much time and effort are also spent subjectively evaluating sperm morphology and morphometry to determine semen quality. Methods used in the staining may cause a slight change in the measurement values of spermatozoa because fixatives can cause cells to shrink a little. When bull spermatozoa are stained for the study of sperm morphology and morphometry, the acquisition of staining affinity by the spermatozoa is associated with structural changes. For conventional sperm morphology and morphometry, these changes are likely to be encountered by sperm fixation to avoid lethal damage from temperature shock before fixation and classifying as either normal or abnormal (Barth and Oko, 1989). The percent of total abnormalities or the percent of specific abnormalities are then used as criteria for assessing semen quality. The association of increased morphological abnormalities of spermatozoa with reduced reproductive efficiency has been reported in bulls (Walters *et al*., 2005). Although, various reports on sperm morphometry of different cattle breeds are reported by Kumar *et al*. (1997); Beletti *et al*. (2005) and on Murrah buffalo by Roy (2014); Javed *et al*. (1997). Meagre information is available on staining techniques and sperm morphometry of Holstein Friesian and Murrah buffalo bulls. Hence, the present work was undertaken to study the sperm morphology by light microscopy and to compare the morphometry of the entire sperm of Holstein Friesian and Murrah buffalo bull using various staining techniques.

MATERIALS AND METHODS

Site

The semen samples were collected from six Holstein Friesian and six Murrah buffalo bulls of Frozen Semen Centre at the Developmental Co-operation of Konkan Limited (DCKL) Aarey colony, Unit No. 6 Goregaon (East), Mumbai. The present study was carried out in the department of Veterinary Anatomy and Histology, Bombay Veterinary College, Mumbai.

Experimental animals

In the present study, six apparently healthy and sexually mature Holstein Friesian and Murrah buffalo bulls aged about four to six years were selected for collection of semen samples. These bulls were reared under the identical feeding and managemental conditions during the entire duration of the study. Bulls were given ad libitum green grass supplemented with concentrate mixture prepared with maize grain, wheat bran, khesari, soybean meal and common salt.

Collection of semen

Semen was collected during morning hours with the help of artificial vagina as per the method suggested by Arthur *et al*., 1982. Prior to collection of semen, all parts of artificial vagina set were cleaned, sterilized and assembled. The inner liner was put into the cylinder and both ends of inner liner were reflected over the cylinder forming water like space between them. The cone along with vial was slipped over one of the ends of the cylinder and then tightened with rubber band. Two third of the outer jacket of vagina was filled with warm water. The temperature inside the artificial vagina was 110^{o}F to 115^{o}F. An air screw was used for blowing air between two layers to create desired pressure. Required amount of lubricant was applied inside the artificial vagina with a glass rod. When the bull was sufficiently excited to jump over the dummy, the penis of the bull was directed into the artificial vagina by holding the sheath to collect the semen in a vial.

Semen examination

A thin smear of neat semen was prepared on a clean glass slide. This smear was then air dried and kept in 5% formaldehyde solution for 30 minutes at 37^{o}C temperature for sperm fixation. Then smears were stained with Haematoxylin Eosin, Toluidine Blue, Silver nitrate as per Culling (1969) and Eosin Nigrosin dyes as per Hancock (1952). Smears were examined under the light microscope. Seven measurements were made including the head length, head width, head base length, acrosomal cap length, acrosomal cap width, mid piece length and total tail length for two hundred spermatozoa with normal morphology. Comparisons were made on sperm morphology and morphometry of Holstein Friesian and Murrah buffalo bull using various staining techniques.

Data analysis

Analysis of the normal distribution of data was done. Statistical comparisons between two breeds were performed with two-way analysis of equal variance (ANOVA) ($P<0.01$). Analysis of the normal distribution of data was examined as per the method of Snedecor and Cochran (1994) for sperm morphometry.

RESULTS AND DISCUSSION

In the present study, the sperm morphology of Holstein Friesian and Murrah buffalo bull

consisted of head, neck, mid piece, principal piece and end piece. (Figure 1 and Figure 4). The head was covered by the acrosome on the rostral and lateral surface and the calyx on the lateral and caudal surface. The neck showed a depression (fossa). Longitudinally segmented columns connected the head to the tail. The middle piece was continuous with the neck anteriorly and posteriorly with main piece and end piece. The sperm morphology of Holstein Friesian and Murrah buffalo bull in the present study was similar to each other and was in agreement with the findings of Banks (1993) in domestic animals and Rougue (2004) in bull.

The comparative sperm morphometrical results in Holstein Friesian and Murrah buffalo bull by using various staining techniques are depicted in Table 1. The findings of mean head length and head width using various stains for spermatozoa of Holstein Friesian bull corroborates with the observation made by Katarzyna *et al.* (2014) in Holstein Friesian bulls and Roy (2014) in Cross bred cattle. The finding of mean head length and head width for spermatozoa of Murrah buffalo bull using various stains was similar to the observation reported by Roy (2014); Ahmed *et.al* (1990) for the same species. The mean head base length, acrosomal cap length and acrosomal cap width of spermatozoa for Holstein Friesian and Murrah bull (Figure 2) was in agreement with the reports of Roy (2014) in cross bred and Murrah buffalo bull respectively. The finding of mean mid piece length for Holstein Friesian bull was close to the observations reported by Shahani *et al.* (2010) in different cattle breeds. The mean mid piece length for Murrah buffalo bull (Figure 3) was similar to the findings made by with Javed *et al.* (1997) for the same species. The mean total tail length of Holstein Friesian corroborates the reports of Kumar *et al.* (2001) in Nagori cattle and Roy (2014) in cross bred while the findings of mean total tail length of Murrah bulls is in line with the finding of Roy (2014); Javed *et al.* (1997) for same species.

The present study on sperm morphology indicates that the Haematoxylin Eosin and Eosin Nigrosin are the suitable staining methods for evaluation of sperm head, Toluidine Blue is suitable stain for the evaluation of mid piece whereas Eosin Nigrosin is suitable stain for the evaluation of acrosomal cap. Appreciable evaluation was not possible in smears stained with Silver nitrate. Also, in the present study, an individual bull variation was not found for the head length, head width, head base length, acrosomal cap length, acrosomal cap width, mid piece length and total tail length of spermatozoa for both Holstein Friesian bulls and Murrah bulls. But the Paired 't' test in equal variance analysis showed that head length, head width, mid piece length and total tail length were highly significant ($P<0.01$), whereas Paired 't' test for head base length, acrosomal cap length and acrosomal cap width were non significant ($P<0.01$). Furthermore, the sperm morphometric characteristics varied substantially between Holstein Friesian and Murrah buffalo. Holstein Friesian sperm is longer in length than Murrah buffalo sperm length.

From the present study, it may be concluded that the sperm morphological characteristics of Holstein Friesian bull are similar to that of Murrah buffalo bull. It may also be concluded that the Haematoxylin Eosin, Toluidine Blue and Eosin Nigrosin are the suitable dyes for staining quality for morphological assessment of spermatozoa.

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Table 1. Comparative sperm morphometry of Holstein Friesian and Murrah buffalo bulls using various stains.

Sr. No.	Parameter	Holstein Friesian bulls	Murrah buffalo bulls	't' value
		mean ± SE	mean ± SE	
A. Haematoxylin Eosin stain				
1	Head length (µm)	9.73±0.09	7.52±0.02	24.69**
2	Head width (µm)	5.10±0.04	4.75±0.09	3.53**
3	Head base length (µm)	2.18±0.05	2.50±0.0	6.64^{NS}
4	Total tail length (µm)	60.83±0.60	56.33±0.21	7.07**
B. Toluidine blue stain				
1	Head length (µm)	9.72±0.08	7.32±0.07	22.01**
2	Head width (µm)	5.05±0.02	4.65±0.09	4.22**
3	Head base length (µm)	2.13±0.02	2.38±0.03	6.71^{NS}
4	Mid piece length (µm)	13.12±0.14	11.50±0.43	3.58**
C. Silver nitrate stain				
1	Head length (µm)	9.68±0.08	7.52±0.03	24.39**
2	Head width (µm)	5.03±0.02	4.57±0.05	8.68**
3	Head base (µm)	2.17 ± 0.03	2.45±0.02	7.06^{NS}
4	Total tail length (µm)	60.85±0.41	55.72±0.16	11.59**
D. Eosin Nigrosin stain				
1	Head length (µm)	9.73±0.08	7.55±0.02	25.03**
2	Head width (µm)	5.12±0.05	4.78±0.10	2.89**
3	Head base length (µm)	2.17±0.03	2.45±0.02	7.06^{NS}
4	Acrosomal cap length (µm)	3.70±0.08	3.57±0.03	1.58^{NS}
5	Acrosomal cap width (µm)	4.50±0.0	4.50±0.03	0^{NS}
6	Mid piece length (µm)	13.27±0.15	10.25±0.17	13.45**
7	Total tail length (µm)	61.37±0.08	55.83±0.21	24.37**

Note: **Highly significant (P>0.01), NS - Non significant (P>0.01).

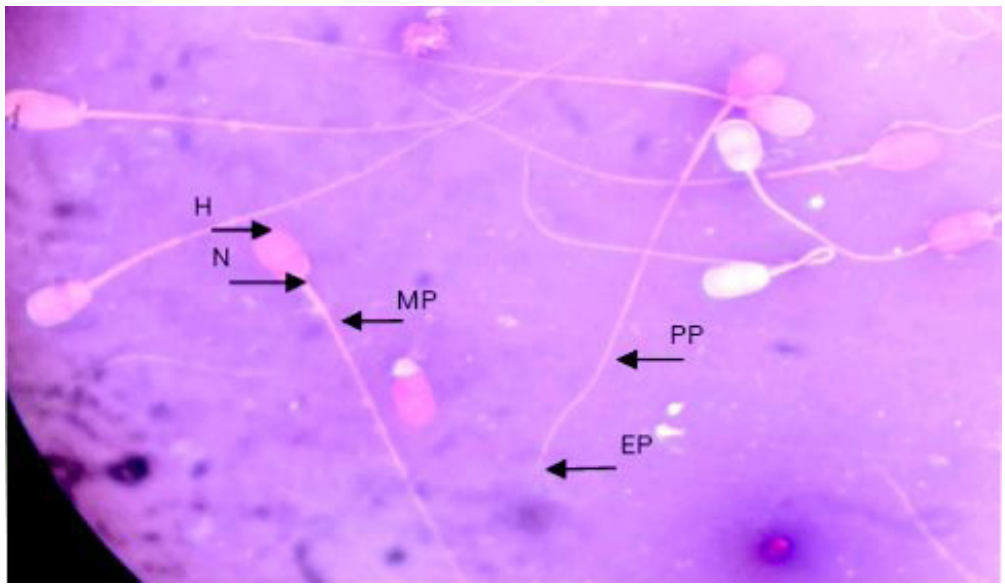

Figure 1. Photomicrograph of mature spermatozoon showing head (H), neck (N), mid piece (MP), principal piece (PP) and end piece (EP).
(Eosin Nigrosin X 1000)

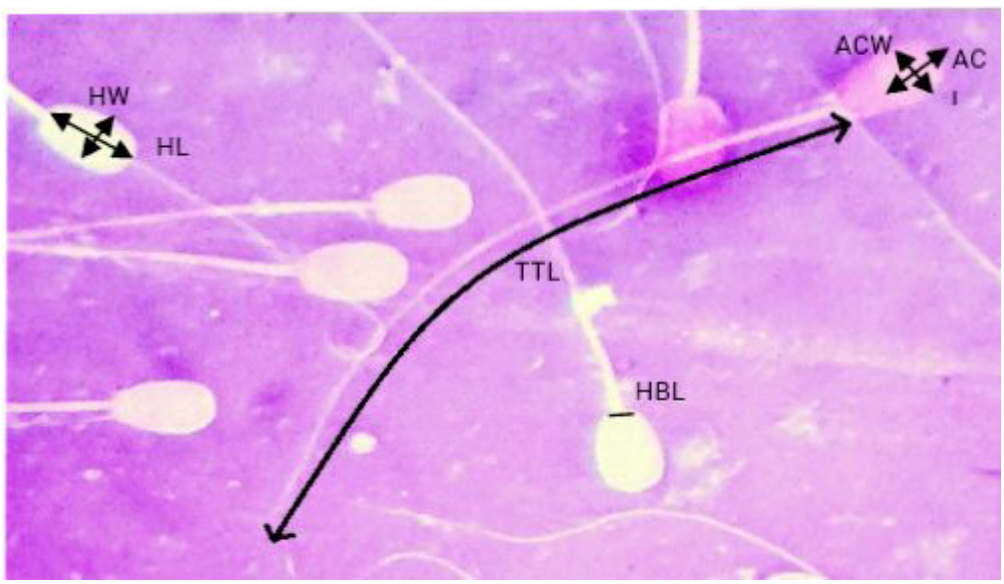

Figure 2. Photomicrograph of mature spermatozoon showing head length (HL), head width (HW), head base length (HBL), acrosomal cap length (ACL), acrosomal cap width (ACW), total tail length (TTL).
(Eosin Nigrosin X 1000)

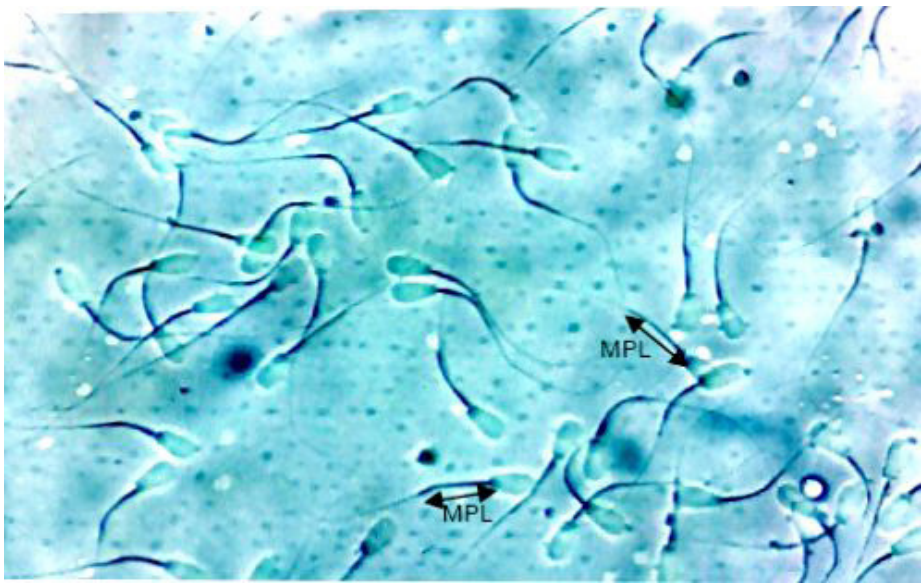

Figure 3. Photomicrograph of mature spermatozoon showing mid piece length (MPL). (Toluidine Blue X 400)

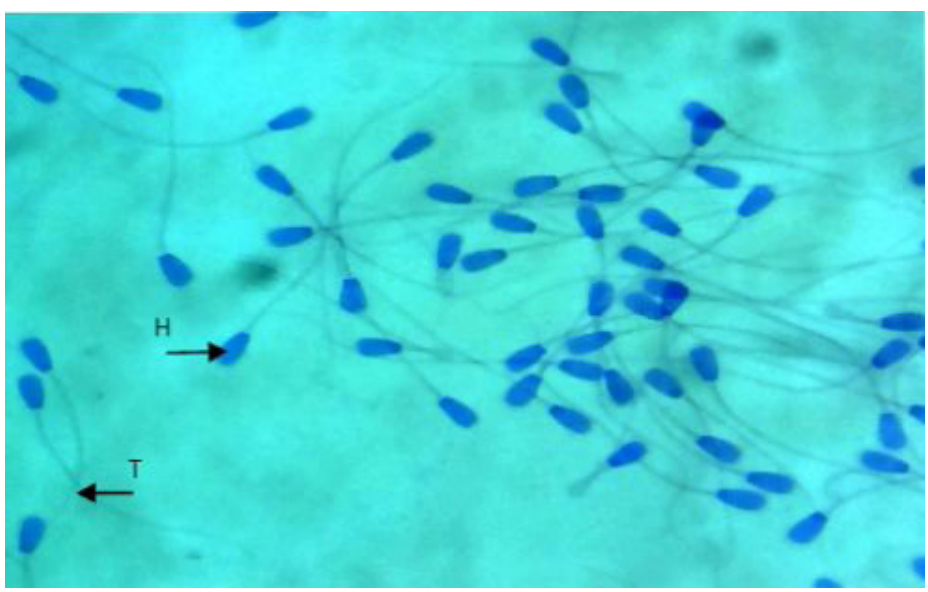

Figure 4. Photomicrograph of mature spermatozoon showing head (H), Tail (T). (Haematoxylin and Eosin X 400)

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