ELECTRON MICROSCOPIC AND SPERM FUNCTION STUDY OF SPERMATOZOA OF HOLSTEIN FRIESIAN BULL

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ABSTRACT

Semen samples were artificially collected from apparently healthy and sexually mature Holstein Friesian bulls (n=6; age, 4-6 yr). Transmission electron microscope revealed mature spermatozoon consisting of head covered by acrosome, neck had a depression continuous to middle piece which consisted of a flagellar core and fibers. The middle piece was followed by principal or main piece and terminated in an end piece. In sperm function test, 90.33±1.20% acrosome were intact and 87.67±2.08% sperms were hypo-osmotic swelling test reactive, thus indicating good quality of semen.

Keywords: Electron Microscope, Holstein Friesian, Micrometry, Spermatozoa, Sperm function

Reproduction is closely linked to semen quality and normal sperm structure. Sperm morphometry, in combination with other objective traits, can be useful for developing a fertility index. An intact plasma membrane is required for the normal sperm functions. In recent years, more attention has been given for evaluating the sperm membrane integrity as it has fundamental importance in fertilization process. Thus, the assessment of membrane function may be a useful indicator of the fertilizing ability of spermatozoa (Nie and Wenzel, 2001). The present study conducted the micrometry of spermatozoon of neat semen by electron microscopy and sperm functional capacity in Holstein Friesian bulls.

For seminal analysis, a group consisting of six Holstein Friesian bulls (age, 4-6 yr) reared under the identical feeding and management conditions were selected. Semen samples were collected during morning hours using artificial vagina and the semen samples were placed in thermoflask with ice cubes and transported to the laboratory within 30 min after collection. The electron microscopy was carried out by using Negative staining. The different dilutions of semen samples were taken on carbon coated copper grids and incubated for 5 min at room temperature. Subsequently, these grids were kept on Whitman paper for drying followed by staining with 2% uranyl acetate for 5 min. The sample grids were washed with distilled water, dried and visualized under Transmission Electron Microscopy (TECNAI 12BT, 120 KV, FEI). Six measurements were made including the head length, head width, length of middle piece, length of main piece, length of end piece and total tail length for two hundred spermatozoa with normal morphology. In sperm function tests, acrosomal damage was assessed by Giemsa staining and Hypo-osmotic swelling test (HOST) was carried out as per the standard method (Nie and Wenzel, 2001). About 100 sperm were counted and expressed in percentage. The analysis of the normal distribution of data for sperm morphometry was carried out as per the standard method.

In the present study, the sperm morphology of Holstein Friesian bull consisted of head, neck, mid piece, principal piece and end piece (Figure 1). The head was covered by the acrosome on the rostral and

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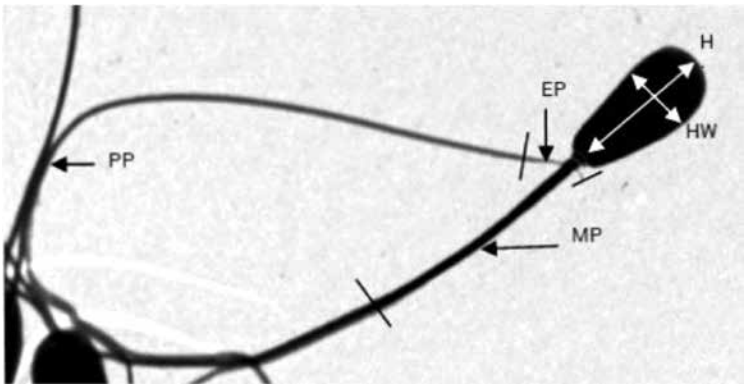

Figure 1: Transmission electromicrograph by negative staining showing spermatozoon of Holstein Friesian bull in neat semen. Head length (HL), Head width (HW), Mid piece (MP), Principle piece (PP), End piece (EP)

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 piece. The middle piece was continuous with the neck anteriorly and posteriorly with main piece followed by end piece. The sperm morphology of Holstein Friesian bull in the present study was in agreement with the findings in bull (Rougue, 2004).

The mean values of head length using negative staining for spermatozoa of Holstein Friesian bull (Table 1), was similar to the findings reported in the Holstein Friesian bull under electron microscopy (Bahr and Zeitler, 1964) and in Holstein Friesian bull under light microscopy (Sardar, 2005). The mean values of head width and mid piece length using negative staining for spermatozoa of Holstein Friesian bull was fairly similar (Table 1), to the observations recorded in the Holstein Friesian bull under light microscopy (Sardar, 2005). On contrary, in an earlier study, a shorter mid piece length was reported in Holstein Friesian under electron microscopy (Bahr and Zeitler, 1964). More recently, a wide variation in the length of mid piece was observed between buffalo bulls of different breeds (Aggarwal *et al.,* 2007) suggesting sperm biometric characteristic might be associated with male fertility. The mean values of length of main piece for Holstein Friesian bull (Table 1), fairly corroborates with the reports in Holstein Friesian under electron microscopy (Bahr and Zeitler, 1964) and Holstein Friesian bull under light microscopy (Sardar, 2005). The mean value of total tail length

Table 1: Sperm micrometry and sperm function attributes of spermatozoa of neat semen of Holstein Friesian (HF) bull

Parameter	HF-1	HF-2	HF-3	HF-4	HF-5	HF-6	Mean±SE
Head length, µm	9.88	9.52	8.9	8.46	8.54	8.64	8.99±0.24
Head width, µm	4.13	4.12	4.5	4.05	3.95	3.8	4.09±0.09
Mid piece length, µm	12.6	12.6	13.6	16.4	13.2	13.0	13.6±0.58
Main piece length, µm	43.6	41.2	42.4	43.7	42.3	43.5	42.8±0.42
End piece length, µm	3.67	3.58	3.24	3.5	3.36	3.63	3.48±0.07
Tail length, µm	59.9	57.3	59.2	63.6	58.9	60.2	59.9±0.9
Acrosome integrity, %	90	95	94	92	89	94	90.3±1.2
HOST reactive sperm, %	85	93	89	90	83	95	87.7±2.1

fairly supports with the result under light microscopy (Sardar 2005). However, another study has shown no correlation between sperm morphology and fertility (Linford *et al.,* 1976), thus clear associations between normal bull sperm morphology and fertility continuing to remain elusive.

In present study, the Holstein Friesian bull sperms having intact acrosomes was higher (Table 1), as compared to earlier findings in Kankrej bulls (Patel and Siddiquee, 2013). In present study, the spermatozoa showing swollen head and coiled tail indicated sperm with intact plasma membrane as observed in Stallion (Nie and Wenzel, 2001). The mean values for HOST reactive spermatozoa of Holstein Friesian bull semen (Table 1), was in agreement with the observation recorded in Murrah buffalo bull (Rasul *et al.,* 2001). The acrosome intact sperm percentage and HOST percentage was >85 % in neat semen, thus, indicating good quality semen.

It can be concluded that sperm morphometric characterization assisted by electron microscope and sperm function test in neat semen of Holstein Friesian bull may provide fast and reproducible measurements to indicate good quality semen.

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